The optimisation of a method for total selenium analysis and application to cereal grain foods

A thesis submitted in fulfillment
of the requirements for the degree of
Master of Applied Science

Elis
B.App.Sci. (Food Science and Technology, Hons)
RMIT University

School of Applied Sciences
Science, Engineering and Technology Portfolio
RMIT University

February 2008

Declaration

I hereby declare that all work carried out in this project was performed while I was enrolled as a postgraduate research student in Applied Chemistry within the School of Applied Sciences, Royal Melbourne Institute of Technology (RMIT) University, City Campus. To the best of my knowledge, this work has not been submitted in whole or part for any other degree or diploma in any University and no material contained in this thesis has been previously written or published by another person, except where due reference is made in the text.

Elis

February 2008

Acknowledgments

I would like to firstly express my special appreciation to my supervisor, Dr Darryl Small. He has kindly provided invaluable advice, support and encouragement throughout my research and during preparation of this thesis. I am particularly grateful for his patience, thoughtfulness and practical approach as well as his guidance which has enabled me to overcome the challenges encountered during my project.

This project was carried out at the City Campus of RMIT University and many people there provided help. I would like to express my gratitude to the staff including Karl Lang, Zahra Homan, Nadia Zakhartchouk, and especially Paul Morrison, for their friendship as well as enthusiastic assistance and support in using the various items of laboratory equipment. Paul contributed greatly by showing me how to set up and use the ICP-MS system. His skill in troubleshooting and maintaining the instrument were invaluable. I also thank the other students past and present in the Food Chemistry group at RMIT University for their friendship, particularly my best friends, Marissa Wijaya and Cecillia Widjaja for their warmth, help and thoughtfulness.

Many people made their valuable time available and provided suggestions. I am grateful to my co-supervisor, Professor Philip Marriott in Applied Chemistry and the Australian Centre for Research on Separation Science, School of Applied Sciences at RMIT University, Melbourne. He kindly provided me with support, suggestions as well as access to instrumental facilities used during all stages of this work. I also thank Dr Jeff Hughes (RMIT University) who helped with the statistical aspects of selenium analysis. Miss Cheau Yin Tan, (Master of Food Science and Technology student) worked with me in the early stages of the methods optimisation studies and I thank her for her contribution.

I gratefully acknowledge the financial support provided by the Australian Government's Endeavour IPRS Award and RMIT University International Research Scholarships (RUIRS) Award throughout the course of this study.

I would like to express my special thanks to my friends in the Campus Christian Movement (CCM), Susan Rusic, Ferry Dharmawan, Pearline Kong and Ting Wang as well as my best friend in Indonesia, Rudy Lee, for their moral support, help and patience which encouraged me to complete this study. I am particularly grateful to my parents and all of my family members in Indonesia for their love, encouragement and understanding.

Publications and presentations

Most of the research reported in this thesis has been presented in various conferences and the details are as follows:

Conference presentations

Elis, Marriott, P. J., & Small, D. M. (2006). *Preliminary study of various methods to analyse total selenium content in bread-mix.* Paper presented at the 56th Australian Cereal Chemistry Conference, Fremantle, 10th-14th September.

Elis, Marriott, P. J., & Small, D. M. (2006). *Comparison and validation of digestion methods for the analysis of total selenium content.* Paper presented at the 14th Annual RACI Analytical and Environmental Divisions R&D Topics, Wollongong, 5th-8th December.

Elis, Marriott, P. J., & Small, D. M. (2007). *The analysis of the effect of bread-making processing steps on total selenium content.* Paper presented at the 10th Government Food Analysts Conference, Melbourne, 14th-16th February.

Elis, Marriott, P. J., & Small, D. M. (2007). *Measuring the Selenium content of cereal foods using ICP-MS.* Paper presented at the 40th Annual AIFST Convention, Melbourne, 24th-27th June.

Elis, Tan, C. Y., Marriott, P. J., Hughes, J. G., & Small, D. M. (2007). *Optimisation and application of total selenium analysis of cereal based foods using microwave digestion and ICP-MS.* Paper presented at the 57th Australian Cereal Chemistry Conference, Melbourne, 5th-10th August.

Abstract

Cereal based foods, including breakfast cereals and Asian noodles are potentially good sources of selenium. Although these are major foods consumed globally, their contribution to dietary intakes of selenium is unclear. In addition, there has been very limited research into the effect of processing steps on the analysis and apparent retention of selenium. The low levels typically present in foods and the presence of multiple chemical forms of the element provide significant analytical challenges to research in this area. Therefore, the aims of this study were firstly to evaluate and validate procedures for extraction and measurement of selenium in wheat flour. Secondly, the procedure has been applied to analysis of selenium in cereal foods. The methods employed were firstly validated using wheat-based reference materials and then samples of various breakfast cereals as well as different styles of Asian noodles were analysed. Selenium was extracted using closed- vessels by microwave digestion with a mixture of nitric acid and hydrogen peroxide, followed by determination through Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). The optimum conditions for selenium determination in cereal based foods involved the digestion of 0.1 g samples using 1 mL of nitric acid and 1 mL of hydrogen peroxide. The addition of 1% (v/v) methanol was found to enhance the sensitivity of the ICP-MS system. Two particular isotopes of selenium (77 and 82) could be effectively employed in the analysis and there was no significant decrease in total selenium in the digested extracts during storage for up to twelve days under refrigeration and room temperature conditions. Good precision levels were obtained and the total selenium levels in the breakfast cereal samples ranged from 0.059 to 0.378 μg/g. For white salted noodles the values varied between 0.057 and 0.712 μg/g, for yellow alkaline noodles, 0.109 to 0.265 μg/g and 0.077 to 0.284 μg/g for fried instant noodles. There was no apparent change observed in total selenium during the processing of fried instant noodles, indicating the effectiveness of the extraction method developed here. It is concluded that microwave digestion is an effective approach to sample extraction, the procedures validated in this study are suitable for cereal grain foods and that there is considerable variation in the selenium contents of breakfast cereal and Asian noodle products.

Table of contents

List of tables

List of figures

Abbreviations

77Se or ^{77}Se	Selenium 77 isotope
82Se or ^{82}Se	Selenium 82 isotope
AACC	American Association of Cereal Chemists
AAS	Atomic absorption spectrophotometry
ANZ	Australia New Zealand
AOAC	Association of Official Analytical Chemists
CCF	Face-Centered Central Composite Design
d77Se	The difference between expected value and measured value for Selenium 77 isotope
d82Se	The difference between expected value and measured value for Selenium 82 isotope
DEDSe	Diethyl diselenide
DESe	Diethyl selenide
DMDSe	Dimethyl diselenide
DMSe	Dimethyl selenide
EAR	Estimated Average Requirement
ED	Exudative diathesis
ETAAS	Electrothermal atomic absorption spectrophotometry
FAES	Flame atomic emission spectrometry
FAO	Food and Agriculture Organisation
GFAAS	Graphite furnace atomic absorption spectrophotometry
GPX	Glutathione peroxidase
HGAAS	Hydride generation atomic absorption spectrophotometry
ICP-AES	Inductively coupled plasma - atomic emission spectrophotometry
ICP-MS	Inductively coupled plasma mass spectrometry
In	Indium
KBD	Keshin-Beck disease
KD	Keshan disease
LRNI	Lower Reference Nutrient Intake

n	The number of replicate analyses used in calculation of individual results
N/A	Not available
NAA	Neutron activation analysis
ND	Not determined
NIST	National Institute of Standards and Technology
PE	Polyethylene
PP	Polypropylene
R^2	Coefficient of determination for a regression line
RAA	Ricegrowers' Association of Australia
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake
RM	Reference Material
RNI	Reference Nutrient Intake
RT	Room temperature
RTE	Ready-to-eat
sd or SD	Standard deviation
Se	Selenium
Se-Cys	Selenocysteine
Se-Met	Selenomethionine
SRM	Standard Reference Material
UK	United Kingdom
UL	Upper Limit
US	United States of America
USDA	United States Department of Agriculture
v/v	Volume per volume
WMD	White muscle disease
XRF	X-ray fluorescence

Explanatory notes

The purpose of these notes is to briefly describe the approaches adopted during the preparation of this thesis. These include the expression of analytical results, as well as the referencing of literature sources:

1. The structures of selenium compounds presented in Chapter 2 have been drawn using CS ChemDraw Ultra® software (version 5.0) supplied by CambridgeSoft Corporation, Cambridge MA;
2. Generally experimental data are presented on a dry weight (or dry matter) basis rather than a fresh weight (or as is) basis unless otherwise clearly specified. The reason that this approach was adopted has been to facilitate direct comparisons of results obtained at different processing stages during manufacture of fried instant noodles. Again, some literature values have also been recalculated to facilitate comparisons;
3. At least triplicate of samples were extracted and each extract was analysed twice for its selenium level. Whenever the results showed poor precision or unexpected values, the samples were re-extracted and re-analysed;
4. Prior to the analysis of sample extracts for selenium contents, an accurately diluted certified standard solution was analysed monitor and ensure the performance of the instrument;
5. In the citation and listing of references and information sources, the style recommended by the American Psychological Association was used and for this purpose EndNote® X software was utilised.

Chapter 1

Introduction

The purpose of this chapter is to provide a very brief overview of the research program described in this thesis which encompasses the analysis and content of selenium (Se) in cereal grain foods including breakfast cereals and three different styles of Asian noodles. The project has been developed on the basis of the following suppositions:

- Cereal based foods are thought to represent a major source of Se in human diets;
- Se is a micronutrient essential to health;
- The amounts present in foods and also those required in the diet are relatively low;
- There is considerable scientific evidence that Se is deficient in the diets of many individuals and animals even in developed countries including Australia. It is likely that many more people are adversely affected in developing countries;
- There have been only limited studies on Se in cereal based foods;
- Few comparative data are available on Se levels in cereal grain foods originating from different countries;
- Breakfast cereals have become one of the most important breakfast choices in the Western diet;
- Asian styles of noodles represent a major end use of wheat with a substantial proportion of total world wheat production used for these products. A number of distinct styles of noodles have long been popular in Asia and more recently in Western countries as well. These include the traditional yellow alkaline types, the white salted styles and the newer instant noodle products; and
- Currently, very few compositional data are available internationally on Asian noodles. In addition, there is virtually no published research into the factors influencing their nutritional quality particularly the impact of formulation and process variables.

Accordingly, this research program has been based upon the hypothesis that there could be differences in terms of Se level in cereal based products manufactured in various countries.

This project therefore seeks firstly to evaluate procedures for the extraction and analysis of total Se, to validate a suitable procedure and then to apply this to a brief comparison of various breakfast cereals and Asian noodle products.

Chapter 2

Background and literature review: the significance, sources, chemistry and stability of selenium

The purpose of this chapter is to provide background and review the relevant scientific literature on Se. The areas covered are the chemistry of Se and its compounds, their nutritional significance, deficiency symptoms and the adequacy of dietary intakes. In addition, changes in compounds under various conditions encountered during food processing and fortification are reviewed.

2.1 Selenium as a nutrient and food component

Se is one of the rarest elements. It was once known only for its toxicity but has come to be recognised for its importance in human and animal health.

During the decade of the 1950s, Schwartz & Foltz (1957) found Se to be a key component of the so-called Factor 3, an active principle found in brewer's yeast able to replace vitamin E in preventing liver necrosis in rats and chickens. This therefore indicated that Se plays a vital role in the metabolism of animals. Furthermore, researchers were able to demonstrate that a variety of enzootic myopathies in cattle and sheep, as well as exudative diathesis in chickens (Patterson et al., 1957) could be controlled effectively by Se, replacing vitamin E.

Se was shown to be an essential component of glutathione peroxidase (GPX), an enzyme that provides antioxidant protection by reducing levels of hydroperoxides in cells (Rotruck et al., 1973). Subsequent findings showed that Se, acting through the expression of a wide range of selenoproteins, has diverse roles in mammals. It is involved in thyroid hormone homeostasis, immunity and fertility, and many other activities in addition to its antioxidant activities. It has also been shown to have anticancer properties, to act as a growth factor and to play important roles in the regulation of synthesis of leukotrienes, thromboxanes, and prostaglandins, as well as other metabolic functions (Imai et al., 1998).

Se levels in foods can vary widely, not only between countries but also between regions in a country. This variation appears to be a result of differing availability of Se in the soils on which an animal is raised or a plant is grown. Typically the major dietary sources in many diets are cereals, meat products, and seafoods. Only small amounts are usually contributed by dairy products, and still less by vegetables and fruits. It is noted that Se levels in cereal grain products can be quite high. According to the surveys performed by Lyons et al. (2004), Se concentration in cereals were ranging from <5 to 750 μg/kg, with most in the 100 to 300 μg/kg range. Much of this variation was associated with spatial variation in soil Se.

2.2 The chemistry of selenium

Se has an atomic weight of 78.96 and its atomic number is 34. It belongs to Group 16/VIA along with oxygen, sulfur, tellurium and polonium, between arsenic and bromine in Period 4 of the Periodic Table of the elements. The chemical properties are intermediate between those of sulfur and tellurium, and its compounds resemble the corresponding sulfur and tellurium compounds in behavior. The outer electronic configuration of Se is $3d^{10}4s^{2}4p^{2}$, with three completely filled inner shells. Its position in the Periodic Table and electronic configuration place Se in the important group of half metals or metalloids, which are neither fully metallic nor non-metallic.

2.2.1 Selenium isotopes

Se has six naturally occurring stable isotopes, ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se. The two most abundant are ^{80}Se (49.8%) and ^{78}Se (23.5%). The availability of these isotopes has enabled researchers to make significant advances in our understanding of the biological roles of Se. They have been particularly useful in the analysis of Se bioavailability (Fairweather-Tait, 1997). The ability to label specific chemical forms of Se with stable isotopes has also helped to understand the role that these different species play in the complex biochemistry of the element (Crews, 2001).

2.2.2 Physical properties of selenium

Se has unique electrical properties. Its conductivity, which is low in the dark, is increased by several 100-fold on exposure to light which also generates a small electrical current in the element. Due to the ability to conduct more easily in one direction than in the other, it is known as an asymmetrical semiconductor (Reilly, 2006).

Elemental Se is very stable and highly insoluble. Under reducing conditions, selenates and other soluble Se compounds that occur in certain soils can be converted into elemental Se and therefore become unavailable for absorption by plants. This process can also remove Se from active recycling and thus reduce the possibility of environmental pollution (World Health Organisation, 1987).

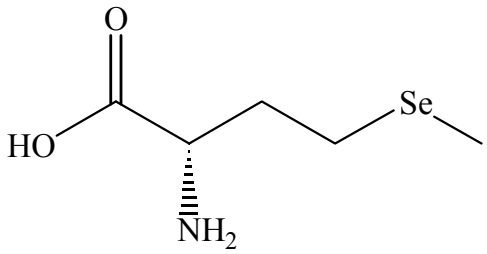
2.2.3 Inorganic compounds of selenium

Like sulfur, Se reacts both with metals and nonmetals, gaining electrons to form ionic compounds containing the selenide ion, Se^{2-}, for example FeSe, Al_3Se_2, and Na_2Se. Se also forms covalent compounds with the other substances. Naturally occurring oxidation states of Se in elemental and combined forms are -2 (e.g. Na_2Se, sodium selenide), 0 (Se, elemental Se), +4 (e.g. Na_2SeO_3, sodium selenite), and +6 (e.g. Na_2SeO_4, sodium selenate) (Reilly, 2006). Se in the +6 state is stable under both acidic and alkaline conditions. This is of significance with regard to the availability for absorption by plant roots in alkaline soils in which selenates naturally occur.

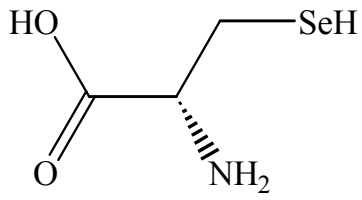
Se forms halides by direct combination with fluorine, chlorine, and bromine, but not with iodine. It also forms oxyhalides, namely oxychloride ($SeOCl_2$), which is a powerful chlorinating agent and oxidant, capable of reacting with other substances explosively.

2.2.4 Organo-selenium compounds

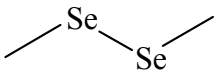
The organic compounds of Se have similar properties, but not identical with organo-sulfur compounds both physically and chemically. Se compounds are less stable on exposure to light or heat and are more easily oxidised than their sulfur analogs due to an increase in atomic number. Some volatile organo-Se compounds, such as dimethyl selenide (DMSE) and dimethyl diselenide (DMDSE) are the result of biomethylation of inorganic Se by microorganisms (Cooke & Bruland, 1987). More complex selenoamino acids (e.g. selenomethionine and selenocysteine) can be found in biological tissues as a consequence of biological pathways by which Se is incorporated into proteins (Jiang et al., 1983). The structure of several organo-Se compounds are displayed in Figure 2.1.



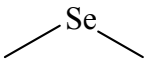
Se-Met (Selenomethionine)



Se-Cys (Selenocysteine)



Dimethyl diselenide



Dimethyl selenide

Figure 2.1 The structure of several organo-selenium compounds

2.3 The symptoms and effects of selenium deficiencies

There are varying symptoms of Se deficiencies which have been recognised. Tables 2.1 and 2.2 show the conditions due to Se deficiency in farm animals and humans respectively.

2.3.1 Selenium responsive conditions in farm animals

White muscle disease (WMD) is a degenerative disease (myopathy) of the striated muscles. It is characterised by lightening of the colour of muscle, which is sometimes accompanied by deposits of calcium. When heart muscles are affected, sudden exertion can cause heart failure and death. If muscles of the limbs are involved, the animal becomes stiff and has difficulty in getting up and down and in walking; hence the other name of the condition given by sheep farmers is "stiff lamb disease" (Oldfield, 1990).

WMD affects many different species, primarily lambs and calves, also horses, goats, poultry, as well as non-farm animals such as rabbits, deer and rats (Wolf et al., 1963). It occurs in all the major sheep-producing countries, including Australia, Canada, New Zealand, Argentina, as well as Britain, South Africa, Scandinavia, Germany, France, Switzerland, Italy, Japan, Ireland and elsewhere. Nevertheless, not all nutritional myopathies in farm animals are Se-responsive. A myopathy due to vitamin E deficiency can occur in both Se-adequate and Se-deficient areas (Peter & Costa, 1992).

Table 2.1 Selenium-responsive conditions in farm animals

Condition	Animals affected	Tissues involved
White muscle disease (myopathy)	Cattle, sheep, poultry, pigs, horses, rabbits, deer, rats	Skeletal, heart, and gizzard muscle
Exudative diathesis	Poultry	Capillary walls
Pancreatic degeneration	Poultry	Pancreas
Liver necrosis	Pigs	Liver
Ill-thrift	Cattle, poultry, sheep	Muscle mass
Still birth, embryonic resorption	Sheep	Embryos
Esophagogastric ulcers	Pigs	Oesophagus, stomach
Sperm immobility	Sheep	Sperm

Source: Oldfield (1990) and Oldfield (2005)

Exudative diathesis (ED) is seen in turkey poults and young ducks. In the chick, it usually occurs in 3- to 6-week-old birds and shows initially as an edema on the musculature of the breast, wings and neck. The growth rate of the birds is also slowed and they lose condition, develop leg weakness, and eventually die (Salisbury et al., 1962). ED has occurred most commonly in commercial flocks fed on low Se grain (Bains et al., 1975).

Hepatosis dietetic (HD), which is also known as liver necrosis or toxic liver dystrophy, is a Se-responsive disease that affects pigs fed on a low vitamin E/ low Se diet. This tends to occur when animals are 3 to 15 weeks years old and can result in a high death rate (Reilly, 2006).

Nutritional pancreatic degeneration is a condition that occurs in poultry fed rations severely depleted in Se. It can develop even when vitamin E levels are high, therefore is an uncomplicated effect of Se deficiency. It leads to degeneration of the pancreas, with development of fibrosis, and causes reduction in the amount of lipase, trypsinogen, and chymotrypsinogen produced. Therefore, the absorption of fat, including vitamin E, is affected, resulting in poor growth and feathering (McDowell, 1992).

Ill thrift is a serious condition in New Zealand and Australia (McDonald, 1975). It is seen particularly in lambs and yearlings, but also occurs in a less severe form in older sheep, beef and dairy cattle on Se –deficient soils. Symptoms vary from a mild, subclinical condition to a severe state, in which there is a rapid loss of weight and even death. A concurrent decrease in wool quality and quantity is seen in sheep (Drake et al., 1960).

Se has been shown to be essential for the efficient and effective operation of many aspects of the immune system. In investigations of the associations between Se and the immune system, Se deficiency was found to distress the function of neutrophil, thereby impairing immune responses (Boyne & Arthur, 1979; Arthur et al., 1981; Arthur et al., 2003). There is also evidence that Se is involved in both the innate, non adaptive system, which includes barriers to infection and nonspecific effector cells such as macrophages, and in the acquired adaptive immune system, in which both T and B

lymphocytes form the major effector cells that mature on exposure to immune challenges (Turner & Finch, 1991).

2.3.2 Endemic diseases related to selenium deficiency in humans

The findings of various Se-responsive conditions in farm animals directed the attention of investigators to the possibility that humans might also be at risk from an inadequate intake of the element. Keshan disease (KD) attracted a great deal of attention in the mid 1930s. Women of childbearing age and children 2 to 10 years old of age are the most susceptible to the disease. The main features of KD include acute or chronic cardiac insufficiency, cardiac enlargement, congestive heart failure, and cardiac arrhythmias (Nezelof et al., 2002). KD is classified into four types: acute, sub-acute, chronic and latent. Depending on which type is present, symptoms can vary widely (Table 2.2).

The etiology of KD remains uncertain. Xie and coworkers (Xie et al., 1964) concluded that the disease was the result of a combination of several factors, one of which is Se deficiency. This view has been further supported by Vanderpas et al. (1990) who found that low levels of Se intakes in Zaire, Africa do not cause KD. This implies that additional factors, besides Se deficiency, are involved; notably Coxsackie B3 virus, a cardiophilic RNA virus. In China, KD has almost been eliminated largely through the provision of selenised salt.

Kashin-Beck disease (KBD), also known as Urov disease, is an osteoarthropathy. It is characterised by chronic disabling degeneration and necrosis of the joints and the epiphysial-plate cartilages of the arms and legs. It becomes evident in childhood and adolescence and leads to varying degrees of disability throughout adult life. Table 2.2 further illustrates the symptoms with advanced cases. Unlike KD, KBD remains prevalent in parts of China, e.g. Shaanxi Province.

Table 2.2 Endemic diseases related to selenium deficiency in humans

Condition	Symptoms/ characteristics	Affected countries	References
Keshan disease	Dizziness, malaise, loss of appetite, and nausea in acute cases to restlessness, and a light dilation of the heart in the sub-acute type.	Some parts of China and Russia	Ge & Yang, 1993; Voshchenko et al., 1992
Kashin-Beck disease	Joint stiffness and pain, possible stunting, various degrees of disability, enlargement of joints and deformity of limbs in advanced cases.	Some parts of China, Russia, Japan and Korea	Diplock, 1987; Mathieu et al., 1997; Research Group of Environment and Endemic Diseases, 1990

Similar to KD, there are other factors that may contribute in the etiology of KBD. These may include iodine deficiency, poor diet, harsh living conditions and intense cold experienced in the prolonged winter of many KBD-endemic areas in addition to Se deficiency (Ge & Yang, 1993). Whilst KD and KBD are the most widely recognised conditions, a variety of other conditions may be associated with Se deficiency.

2.4 The symptoms and effects of selenium toxicity

2.4.1 Selenium toxicity in farm animals

Se toxicity (selenosis) in animals is characterised by general dullness, lack of vitality, emaciation, stiffness and lameness. Horses lose hair from their mane and tail, and cattle from the switch, whilst hoofs became loose and are often sloughed off. There are also reports of teratogenic effects and reduction in reproductive performance (Reilly, 2006).

One of the diseases known as a result of selenosis is “alkali disease” or “blind staggers”. The disease occurs in differing degrees, from a mild, chronic condition, to an acute form resulting in death, sometimes within a few hours of consuming plants containing toxic levels of Se. The animals showed a number of symptoms, including blindness and

paralysis and suffered abdominal pain, with respiratory failure resulting in death. In some cases, loss of appetite as well as extreme pain in the hoofs made the animals unwilling to move about to secure water and food, so that they died of thirst and starvation (McDowell, 1992). Nevertheless, this finding was complicated by other observations done by various researchers. McDowell (1992) further suggested that mycotoxins may play a part in alkali disease as well. The presence of a number of different inorganic elements, namely arsenic, silver and mercury was found to modify the toxicity of Se (Levander & Baumann, 1966). In spite of many years of investigation, the mechanism by which Se exerts its toxic effects in animals has still to be clarified.

2.4.2 Selenium toxicity in humans

In seleniferous areas including North America, Latin America and some part of China, where high Se in urine or serum was observed, tissues most affected by Se toxicity in humans include hair, nails, nervous system and teeth. Hair becomes dry, losing colour, and is easily broken off at the base. A rash develops on the scalp and skin lesions appear elsewhere on the body. Nails become brittle and fall out, and on regrowth are rough and fall out again. A high proportion of those affected develop mottled teeth, in some cases with erosion and pitting (Smith et al., 1936; Bratter et al., 1991; Yang et al., 1983). In China, in the most seriously affected villages, abnormalities of the nervous system and disturbance of the digestive tract have also been observed.

2.5 Required levels of selenium intakes

The levels of Se required in the diet have been determined and many countries have established reference or recommended values. The general term dietary reference value is used to include Recommended Dietary Allowances (RDA) and Recommended Daily Intakes (RDI) (Wenlock & Wiseman, 1993). It is noted that in different countries the terminology adopted has varied, as has the underlying basis for establishment of the specific recommendations. Selected values for Se are presented in Table 2.3 and these demonstrate that different intakes are required by particular groups of individuals depending upon age and gender. During pregnancy and lactation the requirements are also higher (Bender, 1997).

Table 2.3 Dietary Reference Values for selenium for selected countries
(all values are expressed in units of μg/day)

Adults	Australia[1]			UK[2]		USA[3]	
	EAR	RDI	UL	LRNI	RNI	RDA	UL
Men (19 to >70 yr)	60	70	400	40	75	55	400
Women (19 to >70 yr)	50	60	400	40	60	55	400
Pregnancy	55	65	400	N/A	N/A	60	400
Lactation	65	75	400	55	75	70	400

Notes:
EAR = Estimated Average Requirement
RDI = Recommended Dietary Intake
LRNI = Lower Reference Nutrient Intake
RNI = Reference Nutrient Intake
N/A = Not available

Information sources:
[1] NHMRC (2005).
[2] COMA Department of Health (1991).
[3] Food and Nutrition Board (2002).

2.6 Adequacy of selenium intakes

The dietary intakes of Se in different countries based on published results vary considerably, depending on the methods used to assess intakes, as well as on several other causes. These include the difference in food consumption patterns, especially in the types of staple food consumed in addition to representative population samples which might vary from one investigation to another.

A selection of estimated daily Se intakes by adults in a number of countries has been compiled and presented in Table 2.4. These range from as low as 3 μg/day in parts of China where endemic Se deficiency occurs, to as high as nearly 5 mg/day in another part of the same country where selenosis has been reported. Average intakes in Venezuela, although less extreme than levels reported in China, approach the limits of

safe intake. In between these extremes, intakes in many countries appear to be both safe and adequate according to the US Food and Nutrition Board (1980).

2.7 Stability and factors influencing apparent losses of selenium

The accuracy of Se analysis does not depend only on the sample preparation and measurement steps. Sampling and storage affect the reliability of the results in that several processes including volatilisation, adsorption, inter-conversion of species, precipitation or contamination may alter the composition of the sample.

2.7.1 Stability of inorganic selenium compounds

All stability studies have been performed on aqueous samples and adsorption and desorption phenomena were particularly important at the low Se concentrations usually found in environmental samples. Se losses depend on the pH, ion strength, container material and ratio of container surface area per unit of volume. However, light did not have a significant effect on inorganic Se species (Cobo et al., 1994). Se was released from Teflon, polyethylene (PE) and polycarbonate by 50% HNO_3 and less leaching was observed when HCl was used. Optimum conditions for preservation up to 4 months were found for Se(IV) and Se(VI) in natural and distilled water acidified to pH 1.5 with H_2SO_4 and stored in PE or Pyrex containers at room temperature (Cheam & Agemian, 1980). Therefore acidification of samples must be used to prevent precipitation, flocculation or complexation in natural samples, selenite [Se(IV)] being more liable than selenate [Se(VI)] to this type of phenomenon (Oppenheimer & Eaton, 1984). Moreover, the addition of acid increases the ionic strength of the solution, which may minimise the adsorption of Se onto the container walls. Losses of 36% of Se(IV) occurred within 15 days for unacidified samples stored in Teflon containers and were higher than those observed for samples acidified at pH 4 and pH 2, that is 21% and 15% respectively (Gómez-Ariza et al., 2000).

Table 2.4 Estimated selenium intakes (μg/ day) of adults in different countries

Country	Intake (mean or range)	Reference
Australia	55 – 87	McOrist & Fardy, 1989
Bangladesh	63 – 122	Bieri & Ahmed, 1976
Belgium	30	Amiard et al., 1993
Canada	98 – 224	Giessel-Nielsen, 1998
China		
(Eastern urban areas)	53 – 80	Zhang et al., 2001
(Keshan areas)	7 – 11	Combs & Combs, 1986
(Selenosis areas)	750 – 4990	Yang et al., 1989
England	29 – 60	BNF, 2001
Finland	67 – 110	Korpela et al., 1989
France	29 – 43	Ducros et al., 1997
Germany	38 – 47	Oster & Prellwitz, 1989
India	28 – 105	Dang et al., 2001
Ireland	44	Murphy et al., 2002
Japan	104 – 127	Yoshita et al., 1998
Mexico	61 – 73	Valentine et al., 1994
New Zealand (South Island)	19 – 80	Thomson & Robinson, 1993
Poland	30 – 40	Wasowicz et al., 2003
Russia	54 – 80	Aro & Alfthan, 1998
Serbia	30	Djujic et al., 1995
Slovakia	27 – 43	Kadrabová et al., 1998
Turkey	18 – 53	Aras et al., 2001
USA	60 – 220	Longnecker et al., 2001
Venezuela	200 – 350	Combs & Combs, 1986

A combined action of temperature and acidity has been observed to influence the stability of Se(VI). This species remained fairly constant over 1 year for unacidified samples or for samples at pH 4. This contrasts with the results obtained at pH 2, showing a reduction in concentration after 180 days when the samples were stored at room temperature (Dedina & Tsalev, 1995).

A strong temperature-dependence on Se stability has been observed by several authors. Higher losses were found at room temperature compared to storage at 4°C (Cheam & Agemian, 1980). Significant losses of Se(IV) started after 30, 15 and 7 days for samples stored at 4, 25 and 40°C, respectively. A less marked influence of temperature was found for Se(VI), the concentration of which remained fairly constant in samples stored at -20 and 4°C, whereas samples stored at 25 and 40°C displayed a significant reduction in selenate concentration after 6 months and 15 days, respectively. However, a higher stability of both Se(IV) and Se(VI) at 40°C compared to 20°C has been observed and attributed to the increase of the motion of solution molecules reducing their retention on the container walls (Cobo et al., 1994). Optimum preservation of both Se(IV) and Se(VI) stored at -20°C in PE containers during one year has been reported (Cobo et al., 1994).

The stability of inorganic Se in the different container materials decreases as follows: Teflon > silanised glass > borosilicate glass (Pyrex) > quartz > PE > glass (Heninger et al., 1997). A significant reduction in Se (IV) concentration was observed in polypropylene (PP) bottles after 8 months of storage, whereas this species was stable for over 1 year in PE bottles (Cámara et al., 1988). In Teflon containers, the stability of Se(IV) and Se(VI) in water reached a maximum, preservation being possible for 9 months. Under the same conditions, these two species were stable for 2 months in PE bottles at pH 6 and room temperature (Cobo et al., 1994). Moreover, Se(IV) losses of 59, 75 and 81% were obtained for samples acidified at pH 2 and stored in Teflon, PE and PP containers respectively, after 1 year of storage (Gómez-Ariza et al.,1999). However, problems with Teflon materials, such as a partial oxidation of selenite to selenate, have been observed when H_2SO_4 is used for long-term preservation (May & Kane, 1984).

The size of the containers directly influenced the Se stability. Higher losses were observed with larger area per unit of volume (Cheam & Agemian, 1980; Cámara et al., 1988).

2.7.2 Stability of organic selenium compounds

Relatively few stability studies have been carried out on organic Se species. Alkylated Se compounds are volatile and losses by volatilisation may occur, even at room temperature in airtight containers, within one day (May & Kane, 1984). Several types of containers including Teflon, polystyrene and PE of 500mL volume were filled with the working Se (DMSe, DESe, DMDSe, DEDSe) at 4°C and -20°C in the dark and were found to be stable only for 24h. For longer storage times, DESe and DMDSe were more stable than DMSe and DEDSe and no significant losses were observed after 7 days when they were stored in Teflon containers at -20°C (Gómez-Ariza et al., 2000). In contrast, various concentrations of Se-Met from 10 to 100 μg/L were stable in an acidified high ionic strength matrix for a 120-day period of storage in glass and PE containers at 4 and -20°C. However, a significant loss was observed for low level solutions (less than 10 μg/L) stored in glass and PE containers in a low ionic strength matrix (1% HCl in distilled water), showing the influence of both the concentration and type of matrix on the stability of Se-Met (Wiedmeyer & May, 1993).

2.8 Enhancing selenium content in foods

A relatively simple way to achieve an increase in Se levels in food crops is by adding Se to fertilizers. This has been advocated by Rayman (1997) as a practical way of addressing the problem of falling Se intakes in the UK. Arthur (2003) also suggested that it is indeed a convenient and economical way of increasing dietary intake and can be implemented without introducing toxic levels of Se in crops, animals fed on them, or in the human population.

Animal foodstuffs are also enriched with Se. This is achieved by adding Se supplements, both inorganic and organic, to their rations. According to a recent report, these value added products include Se-enriched milk developed in Korea, the “Mega

egg" which has added vitamin E in addition to Se developed in Ireland, as well as Se-enriched chicken and pork (Foley, 2005).

2.8.1 Selenium in functional foods

Se enriched foods have been developed and are promoted to consumers as a convenient way of increasing their Se intake. These products are known as nutraceuticals or functional foods. Several different types of Se-fortified manufactured products have been produced. These include various breakfast cereals, table salt, margarine, sports drink, bread, and other such products (Reilly, 1994; Waitrose, 2005; Laucke Flour Mills Pty Ltd, 2008).

The chemical forms of Se used to fortify foods normally range from inorganic compounds, such as sodium selenite, sodium hydrogenselenite, and sodium selenate, to organic forms, which include selenoamino acids (Se-Met and Se-Cys) as well as Se-enriched yeast. The EU has excluded the use of organic forms including Se-enriched yeast to be used as a supplement due to the fear that an increased intake of selenomethionine could lead to its accumulation in body tissues to toxic levels (Rayman, 2004).

Chapter 3

Background and literature review: procedures for the analysis of total selenium content

The purpose of this chapter is to provide a brief background on the methods available for measurement of total Se. In addition, the specific challenges associated with Se analysis are reviewed.

3.1 Methods available for analysis of selenium

The presence of Se in environmental and biological samples has received increasing attention due to the growing understanding of its essentiality to animal and human health, bioavailability and transport mechanisms. Many different analytical methods have been employed and these have been reviewed extensively (Guerin et al., 1999; Pyrzyńska, 2001; Capelo et al., 2006; Hymer & Caruso, 2006; Reilly, 2006).

A major problem that contributed to the apparent lack of interest in Se displayed by many biological scientists until well into the 20th century was the difficulty they experienced in trying to analyse the element in the materials they studied. This was largely because, in addition to the low levels of Se normally found in biological materials, it is reported to be very volatile and therefore is readily lost during sample preparation. Modern analytical instrumentation, allied with enhanced laboratory procedures and careful quality control as well as the availability of Certified Reference Materials (CRM), has largely overcome these difficulties. Today methods for determining total Se levels are relatively well established (Crews et al., 1997).

3.2 Sample preparation

In most procedures, other than neutron activation analysis (NAA) and X-ray fluorescence (XRF) analysis, the sample must be subjected to certain preliminary steps that usually involve some form of oxidation to remove organic matter and bring the element into the mineralised state in solution (Alt & Messerschmidt, 1988). Reported

approaches include dry procedures, in which the sample is incinerated at high temperature in a furnace or in some other type of heating apparatus in the presence of air or oxygen, and wet digestion, which utilise heating with an acid or mixtures of these.

3.2.1 Dry ashing

The dry procedure or dry ashing is considered to be convenient and versatile despite the risk of losing Se through volatilisation. Moreover, it may take 2-3 days to prepare a solution ready for analysis (Dolan & Capar, 2002). A particular advantage of this method is that it allows for the use of relatively large sample sizes. It also reduces the possibility of contamination of the sample by reagents, since normally only dilute acids are used to dissolve the ash. The use of ashing aid to improve reliability and effectiveness of dry ashing has been reported (Connolly et al., 2004). Excellent recoveries using a programmable electric oven in which samples are incinerated in a series of mineralisation stages for different times and temperatures have also been described (Amaro et al., 1998).

3.2.2 Wet ashing

In this approach, samples are prepared by heating with acids in a "wet" digestion or wet ashing procedure. This is the method of choice in many analytical laboratories because it is applicable to most sample matrices, is rapid and generally has a high recovery rate compared to dry ashing. However, only small sample sizes can normally be used and it also requires relatively large volumes of the digestion fluid, resulting in the possibility of high blanks and sample contamination (Reilly, 2006).

The composition of the digestion fluid used will depend on the nature of the sample to be analysed with nitric acid, or a nitric-sulphuric acid mixture being most frequently used. Hansson et al. (1987) found that the addition of sulphuric acid greatly enhances the oxidation process. However, for certain tissues, nitric-sulphuric acid mixtures may not be fully effective in releasing Se (Tinggi et al., 1992a). The addition of perchloric acid to the mixture has been found to overcome this difficulty, though under certain conditions, its presence may result in the loss of Se through volatilisation (Jones et al., 1975). Additional drawbacks to these conditions can be summarised as follows:

(i) both perchloric acid and sulphuric acid interfere in ETAAS determinations (Welz & Sperling, 1999);
(ii) the presence of perchloric acid at elevated temperatures may create some risk for the operator (Smrkolj & Stibilj, 2004).

Digestion can be carried out in open vessels, involving either a temperature controlled heating block or plate heater and using an appropriate exhaust system to remove fumes. In recent years, closed digestion systems using various digestion apparatus, namely oxygen bomb or microwave-heated sealed polytetrafluoroethylene (PTFE) tubes have been widely adopted. Utilisation of a closed system allows a reduction in the time required for digestion and also in the likelihood of contamination from external sources (Oles & Graham, 1991). As an addition, the systems are adaptable, relatively easily automated and, since it is a closed vessel system, losses due to volatilisation are minimised as well. However, this method is restricted by sample size where reaction of acids with organic sample matrix results in a build-up of pressure due to the evolution of decomposition gases and can cause some problems if safety precautions are not strictly followed. Therefore, it is not suitable for determination of foods containing elements at or near the detection limit (Manjusha, 2007). Nevertheless, this method has been shown to be particularly effective for the preparation of food samples for analysis of volatile elements including Se (Tinggi & Craven, 1996) and is used routinely in many large scale commercial and government analytical laboratories, such as the Central Science Laboratory in the UK (Ysart et al., 1999).

3.2.3 UV-photolysis assisted digestion

UV digestion is the most recently developed procedure for the analysis of Se in food samples. It is reported to be a clean sample preparation method, as it does not require addition of large amounts of reagents. This approach has been applied for destruction and mineralisation of organic compounds in various samples, including sea water (Achterberg & van den Berg, 1994), honey (Buldini et al., 2001), and urine (Philippeit & Angerer, 2001). Furthermore an investigation by Manjusha et al. (2007) has shown good recoveries (94 to 103%) for various commonly consumed food samples including brazil nut, mushroom, curry leaves, pumpkin seeds, mustard seeds, ground nut, pulses,

rice flour, wheat flour, herbal tea, cardamom, ragi flour (*Panicium decompositum)* and beetroot.

3.3 End determination methods for selenium analysis

A variety of analytical techniques have been used for determination of Se in biological materials. Some of the most widely used and generally more practical end-determinations currently available are listed in Table 3.1. The choice of method depends largely on the laboratory facilities and the technical expertise available to the investigators, as well as on the particular object of their study.

3.3.1 Spectrofluorimetry

Spectrofluorimetry is widely used, especially in small-scale studies for the determination of Se in foods and other biological materials. It is based on the reaction of selenites with diamines to produce a piazoselenol which is fluorescent. The most commonly used diamine for the reaction is DAN (2,3-diaminonapthalene). It is a highly sensitive method, which can measure Se concentrations down to nanogram quantities in many different biological matrices and requires only small sample sizes. However, this method is somewhat cumbersome, requires careful supervision and has to a large extent been replaced by other techniques particularly hydride generation atomic absorption spectrophotometry (HGAAS) (Tinggi et al., 1992b).

3.3.2 Atomic absorption spectrophotometry

Atomic absorption spectrophotometry (AAS) in one of its various modes is the most commonly used technique for the determination of a wide range of trace elements, including Se. Its popularity has largely been due to the relatively low cost of the instrumentation required and its use is readily learned by the investigators. Graphite furnace or electrothermal AAS (GFAAS/ ETAAS) are rapid and efficient methods and allow determination of Se in biological materials down to the microgram per gram range with ease (Tinggi et al., 1992a). Problems, however, can be experienced because of matrix interferences, particularly from the presence of phosphates, as well as from excessive volatilisation. According to Tinggi et al. (1992a), the use of HGAAS can avoid such problems. Furthermore, the spectral interferences with GFAAS can also be

corrected by using a form of background correction such as the Zeeman mode (Tinggi et al., 1992b).

Table 3.1 Analytical techniques for trace elements

Technique
Neutron activation analysis (NAA)
X-ray fluorescence (XRF) spectrometry
Atomic absorption spectrometry Flame/ graphite furnace/ hydride generation/ cold vapour (FAAS/ GFAAS/ HGAAS/ CVAAS)
Atomic/ optical emission spectrometry (AES/ OES) with or without inductively coupled plasma (ICP)
Atomic fluorescence spectrometry (AFS)
Mass spectrometry (MS)
Voltammetry:
Differential pulse anodic/ cathodic stripping voltammetry (DPASV/ DPCSV)
Potentiometric stripping analysis (PSA)
Square wave anodic stripping voltammetry (SWASV)
Fluorimetry

Based upon data from Schramel (2000).

3.3.3 Inductively coupled plasma atomic emission spectrophotometry

In the late 1980s, a significant enhancement of analytical capabilities was achieved, in which the acetylene or other gas flame applied in AAS was replaced by plasma discharge for atomisation-excitation in FAES and related instruments. The plasma consists of ions, electrons, and neutral particles formed from argon gas and this operates at far higher temperatures compared to the gas flame of FAES. Inductively coupled plasma atomic/ optical emission spectrophotometry (ICP-AES/ OES) allows simultaneous multi-element analysis and provides similar accuracy to that of ETAAS in many cases. It has become the method of choice in many laboratories as it allows for

analysis of high sample loads in addition to its ability to determine multiple elements in a single sample extract (Reilly, 2006). According to Kumpulainen (1990), early versions of ICP-AES lacked adequate sensitivity to determine concentrations of Se at the very low levels found in some biological samples.

3.3.4 Inductively coupled plasma mass spectrometry

The problem of relatively low sensitivity with ICP-AES has been overcome by the coupling of the ICP with mass spectrometry (MS) in inductively coupled plasma mass spectrometry (ICP-MS). This technology allows the analysis of Se down to levels of 10 pg/g, compared to the μg/g levels achieved using FAAS. However, the equipment is relatively expensive and requires considerable technical expertise for its operation.

In ICP-MS, the ions produced by the plasma are passed through a series of apertures (cones) into a high-vacuum mass analyser. The isotopes of the elements are identified by their mass/charge ratio and the intensity of a specific peak in the mass spectrum is proportional to the amount of the element in the original sample. Several different methods of analysis can be employed depending on the instrument model. The quadripole mass analyser (PQ) is capable of handling high-volume samples and is suitable for the majority of applications, while a high-resolution mass analyser (HRMA) provides higher sensitivities and higher mass resolutions, but with a smaller throughput than PQ (University of Missouri Research Reactor Center, 2004). The dimer from the ICP argon plasma ($^{40}Ar_2$) for the earlier versions of quadripole instrument, has the same mass as the most abundant Se isotope, ^{80}Se, thus precluding measurement of this isotope (Crews et al., 1996). Other less abundant isotopes are usually monitored, including ^{82}Se (9.2%), ^{78}Se (23.5%) or ^{77}Se (7.6%), which have fewer polyatomic interferences from the plasma (Featherstone et al, 2004).

Several other instrumental methods for Se analysis include instrumental neutron activation analysis (INAA), which has the advantage of requiring only minimal sample preparation. It has been shown to be especially useful for determining low levels of Se, for example in human breast milk (Cumming et al., 1992). Even though it requires access to sophisticated equipment, including a nuclear reactor, INAA is recommended by the World Health Organisation (WHO) (1987) as a valuable quality control reference

method against which routine laboratory methods can be evaluated. Other instrumental techniques which are available only in well-endowed laboratories are isotope dilution mass spectrometry (IDMS) and XRF.

3.4 The analysis of selenium in cereal grain foods

Since wheat is an important crop worldwide, it is of special interest to this investigation. In 1949, Se in wheat was analysed using paper chromatography, followed by analysis using electrophoresis in 1962 and ion-exchange chromatography in 1969 (Olson et al., 1970). With the progress of technology, in the early 1990s, Se analysis in cereals and cereal based products was performed using the more advanced tools described in Section 3.3 including spectrofluorimetry (Tinggi et al., 1992b), HGAAS (Tinggi et al., 1992b; Murphy & Cashman, 2001; Yadav et al., in press), ICP-MS (Adams at. al., 2002) and a hyphenated system of HG-ICP-MS (Bryszewska et al., 2004).

Prior to analysis, samples have been prepared by wet acid digestion with various acid combinations as outlined in Section 3.2, either by open or closed vessels. The most recent analysis was performed by adopting microwave technology using mixture of concentrated nitric acid and hydrogen peroxide (Adams et al., 2002) or concentrated nitric acid alone (Bryszewska et al., 2004) as well as a heating block using a mixture of concentrated nitric and sulphuric acids (Yadav et al., in press). Digested solutions are usually treated with HCl to reduce Se(VI) to Se(IV) which is the more stable form of Se.

In reviewing the literature on Se analysis, it is clear that at least four other challenges confront the analysts. These are:

- The relatively low level present in food;
- The existence of multiple forms of Se; these different molecular forms of Se may vary in solubility, stability, and ease of measurement by particular analytical procedures;
- The presence of Se compounds in tissues and foods in forms where they are covalently linked to other components particularly proteins; for example Se-Met which occurs specifically as part of selenoenzymes or non-specifically as part of muscle protein; and
- The difficulty of extracting all of the Se compounds from plant samples due to their high fibre content and high amount of siliceous compounds.

On the basis of the literature reviewed here there is a large range of approaches available for analysis of Se. It also appears that although flour and grain based products have been studied, the specific challenges of selenium analysis in breakfast cereals and instant noodle warrant evaluation in this study.

Chapter 4

Background and literature review: breakfast cereals, Asian wheat noodles and the processing of fried instant noodles as well as their global significance as a food source

The purpose of this chapter is to provide background and review current knowledge on the utilisation of wheat flour for manufacture of instant noodles. The areas covered include the international significance of breakfast cereals and Asian noodles, the different styles of these products, the ingredients and processes applied as well as relevant data on the retention of Se in flour based foods.

4.1 Cereal grains and their classification

Cereals are the fruit of cultivated grasses, members of the monocotyledonous family Gramineae. They are cultivated primarily for human food, livestock feed, seed and as a source material for starch, biofuel and other industries. The cereal crops are wheat, barley, oats, rye, rice (paddy), maize (corn), sorghum, millets and triticale (Kent & Evers, 1994). Among these, wheat, rice and maize are the most important cereal grains produced, consumed and traded around the world. Global production of cereal grains has been approximately 2000 million tonnes over recent years (Food and Agriculture Organisation (FAO), 2007).

There are a number of species of wheat which are grown as crops. The most widely produced are *Triticum aestivum* commonly referred to as bread wheat and *T. durum* or durum wheat, which has particularly hard kernels and is preferred for puffing as well as being used primarily for milling into semolina and flour for macaroni and other pasta products. *T. compactum* or club wheat is also produced in commercial quantities and has very soft kernels. There are numerous genetic varieties (also referred to as cultivars) within each species. In many countries, wheat is classified according to whether it is hard or soft, white or red grained and planted in spring or winter (Fast & Caldwell, 2000).

Following milling, wheat flour can be classified as either hard wheat flour or soft wheat flour which also relates closely with the protein content. Hard wheat flours typically have high protein content (approx 11-15 percent) and are suitable for products requiring stronger structure, including breads and some types of Asian noodles. Soft wheat flours contain approximately 8-11 percent protein and are suitable for products requiring minimal structure including cakes and biscuits. In addition, there are flours having a combination of these quality characteristics, and the wheat is described as having semi-hard grain characteristics. These are used in unleavened breads as well as Asian steamed bread and certain noodles (Bushuk & Rasper, 1994).

Rice or *Oryza sativa*, is one of the leading food crops and is the staple food of over half the global population (Ricegrowers' Association of Australia (RAA), 2007). There are varieties of rice adapted to a wide range of environmental conditions: it can be grown in hot, wet climates, but equally in the foothills of mountainous areas. Rice can be divided into two types: *Japonica* and *Indica*. The former is usually grown in temperate climates, including those in selected regions of Australia, California, Egypt, China and Japan. The grains are relatively round in shape and when cooked, this rice is sticky and moist. *Indica* rice is grown in hot, tropical climates. The grains are long and when cooked, the rice is fluffy and does not stick together. Most of the rice produced in Southern Asia, including India, Thailand, Vietnam and Southern China is *Indica* rice (RAA, 2007). Rice can also be classified as: long grain, medium-grain and short-grain. Medium grain rice varieties are useful for puffing to make breakfast cereal, as well as for brewing adjuncts and for parboiling (Kent & Evers, 1994).

Maize or *Zea mays* was the staple diet of the early native American civilisations – Aztecs, Mayas, Incas and it forms the staple diet in present day Latin American countries and in parts of Africa, Paraguay, Romania and Albania (Kent & Evers, 1994). Similar to other cereal grains, there are a multitude of maize varieties available. The choice of variety will depend on market requirements, environmental conditions, whether the crop is irrigated and the level of disease resistance required.

Cereal grains have a wide diversity of uses in food processing with an increasing range of products becoming available. This reflects the response of manufacturers to demands for new tastes and convenience, the increasing accessibility of travel as well as the trend

to globalisation of food processing. One group of products which continues to see expansion and new developments is the processed breakfast cereals.

4.2 Classification and importance of breakfast cereals

Breakfast cereals falls into two broad categories: firstly those made by a process that does not include cooking and which therefore have to be cooked domestically (hot cereals) and secondly those which are cooked during processing and do not require further cooking. This latter group is commonly known as ready-to-eat (RTE) breakfast cereals. Hot cereals include oatmeal, rolled oats, maize grits and barley meal. Most RTE cereals may be grouped into 12 general categories based on their manufacturing processes: 1) flaked cereals (corn flakes, wheat flakes and rice flakes), including extruded flakes; 2) gun-puffed whole grains; 3) extruded gun-puffed cereals; 6) shredded whole grains; 5) extruded and other shredded cereals; 6) oven-puffed cereals; 7) granola cereals; 8) extruded expanded cereals; 9) baked cereals; 10) compressed flake biscuits; 11) muesli-type products; 12) filled bite-size shredded wheat. RTE breakfast cereals are relatively shelf-stable, light in weight, and convenient for shipping and storage. Primarily, they are made from corn, wheat, oats, or rice, usually with added flavour and sweetening as well as various fortifying ingredients (Kent & Evers, 1994; Fast & Caldwell, 2000).

While hot cereals have been consumed for many years, the development of RTE cereals is relatively recent. They originated in the US in the later part of the 19th century, being developed and used as healthful vegetarian foods in a clinical context (Caldwell et al., 2000). A granulated product, ‘Granula’, made by J.C. Jackson in 1863 may have been the first commercial RTE breakfast cereal. A similar product was made by J.H. Kellogg by grinding biscuits made from wheatmeal, oatmeal and maizemeal. Mass acceptance of RTE cereals was achieved by means of efficient advertising in many countries (Kent & Evers, 1994).

The various stages in the processing of RTE cereals would include the initial preparation of the cereal grains by cleaning, and possibly pearling, cutting or grinding. To these are added selected adjuncts which typically include salt, malt, sweeteners, flavouring materials and sometimes the more heat-stable fortifying agents as well. The

formulation is then mixed with sufficient water to provide a paste or dough of the required moisture content; followed by cooking the mixture; cooling and partially drying, and shaping the material by rolling, puffing, shredding, into the desired form, followed by toasting and packaging. Two general cooking methods are employed; they are direct steam injection into the grain mass in rotating batch vessels and continuous extrusion cooking (Kent & Evers, 1994; Fast & Caldwell, 2000).

RTE cereals have become one of the most important breakfast choices for adults in the US (Siege-Riz et al., 2000). In addition, according to research by Nicklas and co-workers (2002) in New Orleans, La, about 30% of ninth grade students (n=567) have RTE cereals as their breakfast. It was also stated in the same paper that volume sales for hot cereal has increased by 4.3%, 9.8% for breakfast bars during the years from 1998 to 1999. Data from the US Department of Agriculture Nationwide Food Consumption Surveys and from the National Food Supply have shown a 60% increase in consumption of RTE cereals from the year of 1977 to 1994 (McNulty et al., 1996). In another survey of 2,000 American households, a sample population of 603 children aged 4 to 12 years from February 1998 through February 1999 showed that more than 90% of the subjects consumed RTE cereals at least once in the two-week data collection period (Albertson et al., 2003). These and various other surveys show that RTE cereals contribute significantly to the Western diet.

4.3 Classification and importance of Asian wheat noodles

From northern China, Asian noodles were introduced to other Asian countries by traders, seafarers and migrants. Historically, the art of noodle-making has been developing for more than 2000 years. According to the early records, the technology was already remarkably well developed during the Han dynasty which reined in China for the period 206BC-220AD. The products were referred to as 'long life noodles' at that time and their use was reserved for special occasions, particularly birthday celebrations. However, with the introduction of dried noodles during the Sung dynasty (960-1279AD) noodles became more widely consumed on an everyday basis. In addition, a great variety of noodle toppings and tastes had been evolving along with the art and technology of noodle-making (Huang 1996a, 1996b).

Noodles are generally classified on the basis of raw materials. Whilst there are a variety of noodle products made from starch –based ingredients including rice flour and mung bean starch (Collado & Corke, 2004) the primary ingredient of most noodles is wheat flour (Crosbie & Ross, 2004). The other typical ingredients of Asian noodles are salt (sodium chloride) and water. In the case of alkaline noodles, solutions of sodium carbonate, potassium carbonate, sodium bicarbonate or sodium hydroxide, commonly known as lye water or "kan sui" are added. Therefore, depending on the presence or absence of alkaline salts, noodles can be classified as non-alkaline (white salted or commonly known as "Udon" in Japan) or alkaline. Generally, a white, soft and elastic noodle texture is characteristic of white salted noodles (Nagao, 1996). Typical alkaline noodles have bright, clear yellow colour with a firm, chewy texture and a smooth surface (Lorenz et al., 1994). Other ingredients including starches, gums, phosphate salts, and colorants are commonly used for specific effects in both non-alkaline and alkaline noodles. "Soba", a popular noodle consumed in Japan is usually made from a mixture of buckwheat flour and high-protein wheaten flour, water and salt. In addition, other various forms of noodles are available in the market, namely dried noodles, boiled noodles, frozen noodles as well as instant steamed and fried noodles. Most of these products have a rectangular cross-section but may be square or round depending upon the thickness of the noodle sheet and the shape and size of the cutting rolls used (Crosbie & Ross, 2004).

The consumption of Asian wheat noodles is second only to bread globally (Ding & Zheng, 1991). The extent of wheat utilisation in Asian countries is demonstrated in Table 4.1. These data show that annually, the total amount of wheat used for noodles in listed countries is approximately 69.4 million tonnes, representing 12.7 percent of total world wheat production with almost 60% of the wheat products in Asian countries being consumed in the form of noodles (FAO, 2002; FAO, 2004).

Nagao (1998) and Oleson (1998) predicted that wheat consumption would continue to expand in Asian countries over coming decades. The consumption of noodles, particularly instant noodles has also been increasing very rapidly in recent years due to their attractive flavour, convenience and ease of preparation (Anon, 1996; Wu et al., 1998). It is noted that the data presented in Table 4.1 are based upon estimates made in 1989. More recent estimates (Hou, 2001) indicate that the proportions of wheat flour

used in noodle manufacture in most of the countries remain at similar levels while in the cases of Thailand and the Philippines there has been a trend to greater use of flour for noodle production. Furthermore, noodles are now popular in many other countries including Western nations. For Australia, the consumption has been increasing as Asian cuisines have been adopted during recent decades (McKean, 1999).

Table 4.1 The annual consumption of wheat for various Asian countries and the proportions used for noodle production

Country	Annual wheat consumption (million tonnes)	Proportion used for noodle production (percent)
China	107	50-60
Indonesia	1.9	45
Japan	6.3	36
Malaysia	0.84	45
Singapore	0.44	40
South Korea	2.2	40
Taiwan	0.9	36
Thailand	0.37	13
The Philippines	1.6	14
Total wheat consumption in Asia	**121.6 million tonnes**	
Wheat used for noodle production in Asia	**69.4 million tonnes**	
Proportion of wheat consumption as noodle production in Asia	**57.1 percent**	
World wheat production	**546.6 million tonnes**	
Proportion of world wheat production used for noodle production in Asia	**12.7 percent**	

Notes
1 All data apply to the same crop year (1989)
2 World wheat production data is from FAO (2002) and includes all species
3 Data for individual countries (other than consumption from China) were tabulated from Nagao (1995b)
4 Consumption for China was estimated from FAO production and import values (FAO, 2004)

4.4 Basic processing of fried instant noodles

The preliminary processing steps for the manufacture of various styles of Asian wheat noodles are the same (Huang 1996b; Corke & Bhattacharya 1999). The initial processing step involves mixing of flour and all other ingredients to distribute the water uniformly and to produce a crumbly consistency. The resulting material may be rested or mixed for longer periods at low speeds prior to the next stage of processing. The crumbly mass is then compressed in order to produce dough in which the small particles combine to form a single piece. The resultant dough is passed through a set of sheeting rolls which have a suitable gap so that as the mixture is passed between the rolls compression occurs and a dough sheet is formed. Development of the dough occurs during a series of subsequent steps in which the dough sheet is passed through further sets of rolls. These are arranged so that the aperture of each successive set of rolls is smaller. The number of sets of sheeting rolls is typically six or seven. An example of a commercial noodle plant is shown in Figure 4.1, demonstrating how a dough sheet is processed. The resulting dough is a sheet having a thickness appropriate to the style of noodle and the preferences of the consumers. The sheets are then cut into long strands by passing them through one set of rolls which are designed as cutting rolls. An example of these in laboratory scale processing equipment is presented in Figure 4.2.

There are many variations possible following the primary steps in noodle processing as summarised in Figure 4.3. The cut strands may be sold without further processing as fresh noodles. Alternatively, various combinations of drying and cooking processes might be applied. This partly explains the diverse range of noodle products which are available to consumers as mentioned in Section 4.3. Instant noodles have an additional precooking step by steaming prior to frying process.

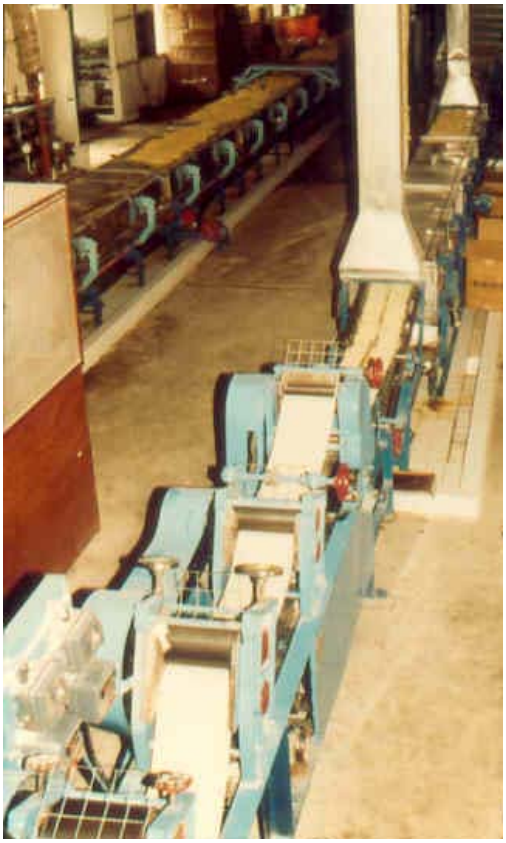


Figure 4.1 A commercial noodle processing plant in Asia showing a dough sheet passing through successive sets of rolls followed by a cutting step and steaming prior to packaging

Figure 4.2 The use of a set of cutting rolls to form noodle strands from a dough sheet using laboratory scale processing equipment

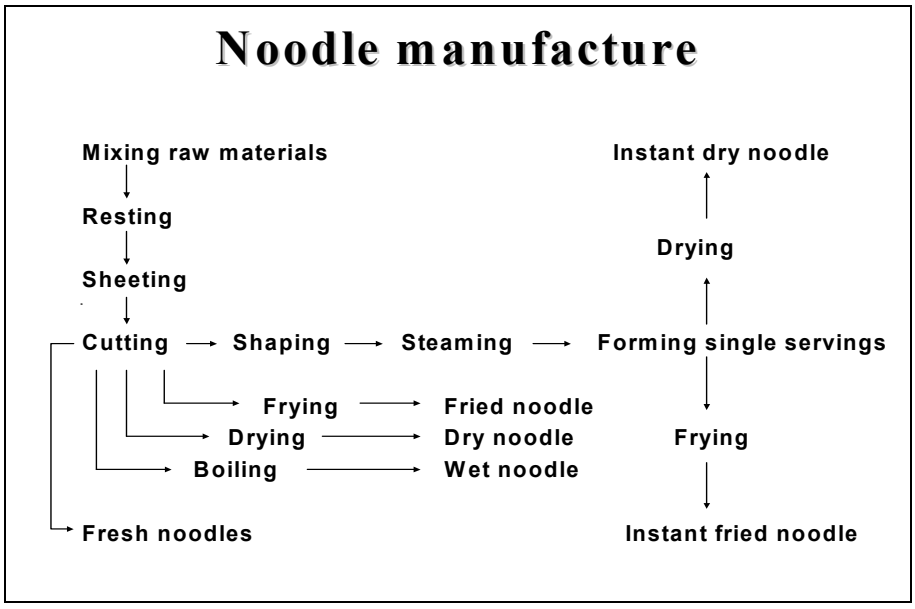


Figure 4.3 Processes used in the manufacture of different forms of Asian noodles
Note Source of information was Hoseney (1994)

4.5 Cereal based foods as sources of selenium

Cereal grains have long been regarded as valuable sources of essential nutrients. They provide energy, protein, minerals and vitamins in the human diet (Kent & Evers 1994). Globally, cereal grain foods are regarded as among the most important sources of Se, even in the absence of fortification. In Australia, it is estimated that nearly half of the dietary Se intake is provided by wheat. In a recent comparative study, Se concentrations in grain were found to range from 5 to 720 μg/kg and it was evident that this wide variation was determined largely by the available soil Se level (Lyons et al., 2005b).

Some of the limited data available for Se content of cereal grain foods from Australia, the UK, the US and New Zealand is presented in Table 4.2. This shows that most US breads and cereals are higher in Se content with an exception for oats where it is lower compared to that from Australia. Overall, the Se content of NZ cereals is lower than those from the other countries. Specifically in relation to the Se status of Asian wheat noodles, no published information could be found.

Table 4.2 Selenium content of several cereal products from Australia, the UK, the US and New Zealand

Cereal products	Australia[1]	UK[2]	US[3]	New Zealand[4]
Oat bran, raw	16.0 – 20.3	ND	45.2	2.00 – 8.80
Wheat flour, whole grain	ND	ND	70.67	
Bread	9.30 – 12.5	4.30 – 9.20	28.2 – 36.6	3.16 – 5.94
Corn flakes	6.29	4.70	8.21	1.77 – 2.00
Muesli	12.8	4.20	17.3	2.21 – 2.52
Oats (cooked)	11.0	3.10	8.12	2.00
Rice (white, cooked)	2.50	13.0	9.31	0
Rice bubbles	1.00	1.70	15.4	0
Pasta (white, cooked)	3.92	4.80	26.4	0.42 – 5.54
Buckwheat flour	ND	ND	5.67	ND

Notes 1 Data tabulated from McNaughton & Marks (2001)
2 Data tabulated from Barclay et al. (1995)
3 Data tabulated from United States Department of Agriculture (2003)
4 Data tabulated from New Zealand Institute for Crop & Food Research Limited (2000)
All data are expressed in unit of μg/100g
ND Not determined

4.6 Current status of selenium research in cereal grains food

A number of analyses have been carried on total Se content in wheat flour and various types of bread in the US (Holden et al., 1991), Ireland (Murphy & Cashman, 2001), the UK (Adams et al., 2002), Australia (Tinggi et al., 1992; McNaughton & Marks, 2002), Romania (Alexiu & Vladescu, 2004), India (Yadav et al., in press), as well as cereals in Finland (Ekholm et al., 2007). Recently, Lyons and coworkers (2005b) have performed research on bio-fortification of wheat as a strategy to increase Se intake in Australia.

Speciation of Se has also been performed and Se-Met was the major form found in wheat (Olson et al., 1969; Tinggi et al., 1992).

Although there are some data on the Se contents of cereal grain products (Table 4.2), there is very limited data found on breakfast cereal products in Australia. In addition, no data have been found from any sources for Asian wheat noodles including fried instant noodles. There is no evidence indicating to what extent either naturally occurring or Se compounds added through bio-fortification or food enrichment might be retained during processing and cooking of these widely consumed products. On this basis, the overall purpose of the current project has been to study the total Se contents of various breakfast cereals and Asian wheat noodle products.

Chapter 5

Summary of background and description of the project aims

The purpose of this chapter is to summarise the context in which this project has been developed and to describe the aims of the project.

5.1 Summary of current situation and significance of the project

Se is a food component essential to health and also contributing to well-being. It has roles in key oxidative reactions and is an integral component of certain enzymes. Se compounds are therefore of significance in antioxidant functions in the body, providing protection from various degenerative diseases including cancers (McNaughton & Marks, 2002; Reilly, 1998). The amounts present in foods and also required in the diet are relatively low, posing a problem in analysis of foods. Further issues are the evidence that intakes of Se in excess of requirements may present risks with an apparent margin between deficiency and excess intakes being relatively narrow. It is also reported that there is considerable variation in the levels of Se in particular food products due to environmental factors (McNaughton & Marks, 2002; Tinggi, 2003). Substantial scientific evidence is available indicating that Se is deficient in the diets of many individuals and animals even in developed countries including Australia. It is likely that many more people are adversely affected in developing countries.

Cereal based foods, including breakfast cereals and Asian noodles are potentially good sources of Se. The research and surveys performed by Siega-Riz et al. (2000), Nicklas et al. (2002), McNulty et al. (1996) and Albertson et al. (2003) have demonstrated that breakfast cereals have become one of the most important breakfast choices in the Western diet. On the other hand, Asian styles of noodles represent a major end use of wheat with more than one twelfth of total world wheat production used for these products. A number of distinct styles of noodles are popular in Asia and more recently have become more widely consumed in Australia as well as other Western countries. These include the traditional yellow alkaline types, the white salted styles and the newer

instant noodle products (Ding & Zheng, 1991; Anon, 1996; Wu et al., 1998; McKean, 1999; Crosbie & Ross, 2004).

Currently, few compositional data are available internationally on Asian noodles. In addition, there is virtually no published research into the factors influencing their nutritional quality, the impact of formulation and process variables. There has been restricted investigation into Se level in cereal based food originating from different countries.

5.2 Hypothesis

Accordingly, this research has been based upon the hypothesis that there could be differences in terms of Se level in cereal based products manufactured in various countries.

5.3 Project aims

The aims of this project are:

1. To develop and optimise a method for Se extraction from cereal grain samples and to establish measurement by ICP-MS. In this context it is necessary to consider the potential loss of Se during extraction as well as factors enhancing the measurement of total Se by ICP-MS.
2. To examine total Se in commercial breakfast cereals and Asian noodles products from various countries.
3. To consider the apparent effects of processing steps on Se analysis and apparent retention in fried instant noodles.

Chapter 6

Materials and methods

The purpose of this chapter is to describe the chemicals, reagents, equipment and methods used during this study. This includes procedures applied in the sampling and preparation of noodles, methods for extraction procedures and analytical methods, and a method for fat analysis along with details of calculations for total Se.

6.1 Materials

The chemicals and reagents used for extraction procedures and fat analysis were of analytical grade or of the highest purity available, unless otherwise specified. Se was used as chemical standards for analytical purposes as well as for spiking purposes. In addition two reference samples were used for method validation and in the assessment of sample extraction procedures. The details of the Se compounds and other chemicals used in the analysis of Se contents are presented in Table 6.1. Details for the reference samples used in the study are presented in Table 6.2.

Table 6.3 shows the details of commercial flour used together with the respective suppliers. The materials used in the analysis of fat contents are provided in Table 6.4. The commercial breakfast cereals examined together with the respective ingredients list and suppliers are listed in Table 6.5. Commercial noodles were purchased from various retail outlets in Melbourne. Based upon the appearance of the products as well as the information provided on the packages, each was categorised according to the three styles of noodles. The details of the samples are presented in Tables 6.6–6.8.

Table 6.1 Details of chemicals and suppliers used for total Se analysis

Supplier	Chemical(s)
MERCK, Australia	Nitric acid 65% Suprapur® (Art. 1.00441, ZU620441)
Ajax Chemicals, Melbourne	Hydrogen peroxide (A260-2.5L PL, UN No. 2014, Batch no. AF512216)
Scharlau Chemie, Spain	Methanol (ME 0310, UN No. 1230)
AccuStandard, USA	ICP-MS Internal Standard - Indium (ICP-MS-IS-IN-10X-1, A7075059), Se ICP Standard (ICP-51N-10X-0.5, B4035066)

Note Description presented as chemical name (product number, batch or lot number)

Table 6.2 Details of NIST (US) reference samples used for this study

Samples	Description	Certified value (µg/g)
Durum wheat flour	RM 8436	1.23 ± 0.09
Wheat flour	SRM 1567a	1.1 ± 0.2

Note Description presented as product number, batch or lot number

Table 6.3 Description of flour sample used for this study

Flour	Description	Supplier
Laucke bread mix	Bio-Fort Se Packed on 02.03.2006, code 1760720	Laucke Flour Mills, Strathalbyn, South Australia

Table 6.4 Details of chemicals and suppliers used for fat analysis

Supplier	Chemicals
MERCK, Australia	Diethyl ether (6.10094.2500, UN No. 1155, Batch No. 38610)
Ajax Chemicals, Melbourne	Hydrochloric acid (A256-2.5L PL, UN No. 1789, Batch no. AA505038), Hexane fraction (A251-2.5L GL, UN No. 1208, Batch No. AH701048)

Note Description presented as chemical name (product number, batch or lot number)

Table 6.5 Description of samples of commercial breakfast cereals

Brand	Ingredients (as listed)	Country of origin
Home brand Processed bran	Wheat bran (64%), wheat flour (gluten), sugar, salt	Australia
Kellogg's All bran	Wheat bran (84%), sugar, barley malt extract, salt, vitamins (riboflavin, folate, thiamin), mineral (iron)	Australia
Kellogg's Corn flake	Corn (90%), sugar, barley malt extract, salt, vitamins (vitamin E, vitamin C, niacin, riboflavin, thiamin, folate), minerals (iron, zinc oxide)	Australia
Kellogg's Nutrigrain	Cereals (44%) (wheat flour, oatmeal, maize flour), sugar, wheat gluten, molasses, salt, barley malt extract, minerals (calcium carbonate, iron), mineral salt (sodium bicarbonate), natural colour (paprika, turmeric), vitamins (vitamin C, niacin, thiamin, vitamin B6, riboflavin, folate)	Australia
Kellogg's Rice bubbles	Whole white rice (89%), sugar, salt, barley malt extract, vitamins (vitamin C, niacin, riboflavin, thiamin, folate), minerals (iron, zinc oxide)	Australia
Kellogg's Special K	Cereals (62%) (rice, wheat), wheat gluten, sugar, wheat flour, minerals (calcium carbonate, iron, zinc oxide), salt, barley malt extract, vitamins (niacin, vitamin B6, riboflavin, thiamin, folate)	Australia

Table 6.5 Description of samples of commercial breakfast cereals (continued)

Brand	Ingredients (as listed)	Country of origin
Nestlé Cheerios	Cereal grains (whole grain corn (22.1%), whole grain oats (19.6%), whole grain barley (16.4%), whole grain wheat (12.1%), whole grain rice (3.8%), rice flour (3.6%)), sugar, partially inverted brown sugar syrup, wheat starch, minerals (calcium carbonate, trisodium phosphate), salt, palm oil (antioxidant (306)), colours (caramel III, annatto extracts), vitamins (vitamin C, niacin, thiamin, riboflavin, vitamin B6, vitamin E, folic acid), minerals (iron oxide, zinc oxide)	UK
Sanitarium Puffed wheat	Puffed wheat	Australia
Sanitarium Weet-Bix	Whole grain wheat (97%), raw sugar, salt, barley malt extract, minerals (zinc gluconate, iron), vitamins (niacin, thiamin, riboflavin, folate)	Australia
Uncle Toby's Bran plus	Wheat bran, sugar, malt extract, mineral (calcium carbonate), salt, vitamins (ß-carotene, vitamin B1, E, B2, folate)	Australia
Uncle Toby's Traditional oat	Rolled oat	Australia
Vogel's Ultra bran	Cereals (79%) (wheat bran, wholemeal wheat flour, corn starch (hi-maize)), sugar, linola (linseed) meal (8%), soy isoflavone concentrate (1.3%), minerals (calcium, zinc), salt, vitamins (vitamin E, folate), natural colour (160b)	Australia

Table 6.6 Description of samples of commercial white salted noodles

Brand	Ingredients (as listed)	Country of origin
Couple soba	Wheat flour, buckwheat flour, yam flour, salt, water	Korea
Gold Star Hokkien noodle	Wheat flour, water, natural colour E100, preservatives (300) (281), canola oil for coating	Australia
Hakubaku soba	Wheat flour, buckwheat flour, salt, water	Australia
Hakubaku somen	Wheat flour, salt, water	Australia
Itsuki yaki-soba	Wheat flour, water, soy bean oil, salt	Japan
Samlip udon	Wheat flour, water, salt, tapioca starch, organic acid	Korea
Sing Lin	Wheat flour, salt, colour (102)	Taiwan
Six Fortune	Wheat flour, salt, water	Taiwan
Tak On Shanghai noodle	Wheat flour, water added, salt, mineral salt (339, 450), preservative (202)	Australia
Wokka	Water, wheat flour, canola oil, corn starch, salt, acidity regulator (575), colours (102, 110)	Taiwan

Table 6.7 Description of samples of commercial yellow alkaline noodles

Brand	Ingredients (as listed)	Country of origin
Double Merinos egg noodle	Wheat flour, water, egg, lye, colour (110)	Australia
No. 1 Foods fresh ramen	Wheat flour, water, salt, preservative (281), mineral salts (500, 341, 501), colour (171)	Australia
Tak On egg noodle	Wheat flour, fresh egg, water added, wheat gluten, mineral salt (500, 501), colour (102, 110), preservative (223)	Australia

Table 6.8 Description of samples of commercial instant noodles

Brand	Ingredients (as listed)	Country of origin
Indomie	Wheat flour, edible vegetable oil, salt, potassium carbonate, sodium polyphosphate, natural gum, sodium carbonate, and tartrazine Cl 19140	Indonesia
Maggi	Wheat flour, vegetable oil [vegetable oil, antioxidant (306 (contain soy))], iodised salt, mineral salts (451, 501, 500), vegetable gum (412), minerals (iron, zinc), vitamins (niacin, thiamin, riboflavin, folic acid)	Australia
Nissin	Wheat flour, tapioca starch, palm oil (contain antioxidant (306)), salt, mineral salts (500, 501)	Hong Kong
Samyang	Wheat flour, palm oil, potato starch, salt, mineral salt (501)	Korea
Trident	Wheat flour, palm oil (antioxidant (320, 321)), salt, mineral salts (500, 501), vegetable gum (466)	Singapore
Trident	Wheat flour, palm oil, salt, edible gum (412)	Thailand
Wei Lih	Wheat flour, tapioca starch, refined palm oil, salt, antioxidant (306)	Taiwan

6.2 Apparatus and auxiliary equipment

The items of equipment used, together with the details of manufacturers and model numbers are presented in Tables 6.9 to 6.11. Table 6.9 provides information on the general laboratory instrumentation used in this study, while the microwave digestion system and ICP-MS systems are detailed in Tables 6.10 and 6.11, respectively.

Table 6.9 Description of equipment and instrumentation

Equipment	Manufacturer/supplier	Model no
Microwave-Accelerated Reaction System (MARS)	CEM Corp., Australia	Mars X Serial no. MD7715 (see also Table 6.10)
ICP-MS	Agilent Technology, USA	Agilent 4500 series 300 G1820A Serial no. 3622J00825 (see also Table 6.11)
Kenwood mixer	Kenwood Ltd, Britain	KM210, Serial no. 0309397
Noodle maker	Domestic 'spaghetti machine' Imperia, Italy	MOD 150, design no. 1048534
Cutting attachment for noodle maker	Imperia, Italy	MOD 150
Kambrook deep fryer	Kambrook distributing Pty Ltd. Australia	KD 53
Oven	Watson Victor Ltd., Australia	Model H23S Serial no. 8590.15
Digestion block	Selby Scientific, England	Type BT5 Serial no. 74078
Heating plate	Industrial Equipment & Control Pty. Ltd., Australia	C.5. 76083V Cat. No. 2090.001
Homogeniser	Breville, China.	BCG300
Grinder	Falling Number AB, Sweden	Model 3100
pH meter	Hanna Instrument, U-Lab instrument, Australia	pH 211 microprocessor pH meter
Weighing balance	N.G. Brown & Assoc. Pty. Ltd., Switzerland	PB303DR SNR 1113051929

Table 6.10 Description of MARS system components

Equipment	Manufacturer	Model no
Pressure sensor	CEM Corp, USA	ESP-1500 Plus
Teflon vessels	CEM Corp	Omni™ D S
Temperature sensor	CEM Corp	RTP-300 Plus Serial no. TFC1762E

Table 6.11 Description of ICP-MS system components

Equipment	Manufacturer	Model No
Auto Sampler	CETAC Technology, USA	ASX 500 Serial no. 020115ASX
Data handling system	Agilent Technology, USA	ICP-MS Chem St. Version A.01.02 Product no. G1834A
0.22μm cellulose ester syringe filters	Filtamate, Australia	Cat. No. FM25220A

6.3 Laboratory procedures for manufacture and processing of instant noodles

In studies of noodles prepared in the laboratory, two batches of noodles were made and the samples of these batches were analysed at least in triplicate. The averages of the results from the analysed data were calculated and are presented in this thesis, representing the value for each batch of noodles prepared.

Instant noodle samples were prepared using procedures based on those described by Bui & Small (2007). The method for making instant noodles involved mixing, sheeting, cutting, steaming and frying. Further details are as follows:

Ingredients used for instant noodles

The basic ingredients used to make instant noodles were: 200.0 g Laucke flour, 80.0 g water, 2.0 g sodium chloride, 0.24 g potassium carbonate, 0.16g sodium carbonate. The oil used to deep fry the noodles was palm oil (Auroma Pty Ltd, Australia).

Method for instant noodles

The procedure for making instant noodles was the same as that for yellow alkaline noodles at the mixing and rolling steps. However, following these, the resultant sheet was not rested but was immediately passed a further four times between the rollers to reduce the sheet thickness before cutting into noodle strands for the further preparation steps of steaming, frying and draining.

Mixing: The salt and the mineral salts were first dissolved in the water and this solution was added to the flour over a period of 30 s in a Kenwood mixer set on speed one. Timing of mixing then began when all the liquid had been added. The mixer was set at the lowest setting (speed 1) for 1 min then it was stopped so that the dough material adhering to the bowl and beater could be scraped down. After that, the speed of the mixer was increased smoothly to setting 4 and allowed to mix for a further 4 min. After a total of 5 min mixing (1 plus 4 min), the resultant dough had a crumbly consistency similar to that of moist breadcrumbs.

Rolling: The dough was first formed into a dough sheet by a process of folding and passing the crumbly dough through the rollers of the noodle machine several times. For this combining step the rollers were set at the maximum gap available, corresponding to 2.7 mm. Typically three passes were required although up to 5 passes were used where necessary in order to give a uniform sheet which held together as a single dough piece. Then this combined sheet was allowed to rest for 30 min. The sheet was sealed in a plastic bag to prevent moisture loss after resting. The thickness of the sheet was reduced stepwise by passing between the rollers of the noodle machine. The roll gap settings used were: 2.2, 1.8 and 1.4 mm.

Cutting: The sheet was cut into strands using the cutting roll attachment of the noodle machine having a cutting width of 2.0 mm. The noodle strands were then cut into 25 cm lengths using a knife before steaming.

Steaming: fresh noodles strands were placed in a steamer and steamed over vigorously boiling water for 2 min. Then they were removed from the steamer and placed onto dry paper towel for 30 s.

Frying: The noodles from the steaming step were then immediately placed into a wire basket and deep fried in palm oil for 45 s. The temperature of the oil was carefully checked and noodles were only placed into the oil once it had attained 150°C.

Draining and cooling: The fried noodles were removed from the oil using the wire basket and allowed to drain for about 30 s. Noodles were then transferred to absorbent paper and allowed to cool prior to placing into a sealed bag or container for storage.

6.4 General methods for characterisation of noodle samples

In the analysis of all samples, multiple analyses were carried as described for the individual analysis procedure. In all cases the results for at least duplicate measurements of individual samples were assessed statistically and are reported as the mean value ± standard deviation. In reporting data, the latter is abbreviated as sd and the number of replicate determinations is referred to as n.

6.4.1 Moisture determination

The moisture contents of samples (flours, dough, steamed noodles, and fried noodles) were measured following the standard air oven method of the Association of Official Analytical Chemists (AOAC) (AOAC, 1990a). For each sample analyses were carried out in duplicate. It is noted that samples were not ground prior to analysis. Empty aluminium moisture dishes with lids were first placed into a pre-heated oven set at 130 ± 3°C. After 1 h, the empty dishes were taken from the oven and cooled in a desiccator containing active silica gel desiccant for a period of 20 min and then weighed. Sub-

samples (approximately 2.0 g) were accurately weighed into the pre-weighed dishes. Then the covered dishes containing the samples were placed into the oven with the lids placed under the respective dishes and dried at 130 ± 3°C. The process of drying, cooling and weighing was repeated after 1 h until a constant weight was attained. The loss in weight was used to calculate the moisture content of the samples using the following equation:

$$\text{Moisture content (percent)} = \frac{\text{Loss in weight of dish, lid and sample upon drying}}{\text{Initial weight of sample}} \times 100$$

6.4.2 Measurement of the pH of noodle samples

The pH values of flour and noodle samples were determined by the AACC standard procedure (AACC, 1994b). For this, a sample (10 g) was thoroughly blended in 100 mL of distilled water using a Breville homogeniser. The mixture was then allowed to settle for approximately 30 min after which the supernatant liquid was decanted and tested with a calibrated pH meter. All analyses were carried out in triplicate.

6.4.3 Measurement of fat content of laboratory noodles

The fat contents of noodles made in the laboratory were determined by acid hydrolysis following the procedures described by James (1995). Firstly, duplicate of samples were ground and weighed accurately (approx. 2 g) into clean, dry Mojonnier tubes. HCl (6 M, 15 mL) was added into the tubes which were then placed in a boiling water bath for 30 mins. After that time period elapsed, the tubes were cooled to room temperature by immersion in a beaker of cool tap water. Following that, diethyl ether and petroleum spirits (25 mL of each) were added into the cooled tubes. Each tube was shaken gently and the pressure build up was released frequently. The upper layer was transferred to a pre-weighed dry evaporating dish and evaporated using a steam cone, followed by drying in the oven at 100°C and cooling in dessicator every half an hour until constant weight was achieved.

6.5 General sampling and extraction procedures used in the analysis of total Se

6.5.1 Preparation of samples for total Se analysis

For analysis purposes, whole meal bread-mix flour, breakfast cereals and noodle products were prepared as follows:

Drying

All of the wet noodle samples investigated were first dried using the air oven method (AOAC, 1990a) as described in Section 6.4.1.

Grinding

All the samples analysed, including commercial products as well as those prepared in the laboratory were ground prior to extraction of Se using a either a domestic coffee grinder (for noodles and breakfast cereals) or the Falling Number 3100 mill (for the Laucke bread mix).

6.5.2 Extraction of Se from flour, breakfast cereals and noodle samples

Microwave extraction

Approximately 0.1 g of sample was weighed into the Teflon-lined Heavy Duty Vessels together with 1 mL of nitric acid, 1 mL of hydrogen peroxide and 8 mL of milli-Q water. The vessels were then sealed and heated according to the recommended temperature/ time settings (Table 6.12) from room temperature to 175°C in three stages.

After digestion, the solutions were allowed to cool for approximately 1h, made up to 25 mL with milli-Q water, filtered through 0.22 μm cellulose ester filters and measured on the ICP-MS system.

Table 6.12 Microwave settings

Stage	**1**	**2**	**3**
Power (W)	1200	1200	1200
Temperature (°C)	100	150	175
Heating time (min)	5	5	3
Holding time (min)	5	5	15

Digestion block extraction

Approximately 0.1 g of samples was weighed into glass tubes together with 1 mL of nitric acid, 1mL of hydrogen peroxide and 8mL of milli-Q water. The vessels were then covered and heated at 84 ± 3°C for 4 to 5 h.

Following the digestion, the solutions were allowed to cool for approximately 1 h, made up to 25 mL with milli-Q water, and filtered through 0.22 μm cellulose ester filters before being measured on the ICP-MS system.

6.6 Procedures and calculations applied generally in the analysis of total Se

In the analysis of total Se, no storage of partially treated samples was required. For all sample extracts, at least duplicate determinations were carried out on the same day.

6.6.1 Preparation of solutions

Preparation of internal standard

Indium stock solution *(1 mg/L):* 1 mL of the standard solution (100 mg/L) as purchased was made up to 100 mL in 2% nitric acid. This solution was used as internal standard and was stable for approximately 6 months at room temperature.

Preparation of standard Se

Standards of Se were prepared at 6 different concentrations which included: stock solutions (1 mg/L), standard solution I (2 μg/L), standard solution II (5 μg/L), standard solution III (10 μg/L), standard solution IV (15 μg/L), and standard solution V (20 μg/L). To each standard solution (I to V), methanol and internal standard were added and Table 6.12 presents the amounts added to each standard solution.

Se stock solution *(1 mg/L):* 10 μL of the purchased standard solution (10000 mg/L) were made up to 100 mL in 2% nitric acid. This solution was used to prepare the standard solutions and was stable for approximately 6 months at room temperature.

***Se standard solutions**:* stock solution, methanol, and internal standard were pipetted into 25 mL volumetric flasks and made up to the mark with nitric acid (2% v/v). These solutions were freshly prepared immediately prior to use. The amount of each solution added to the standard solutions is presented in Table 6.13.

Table 6.13 Se standard solutions used for ICP-MS

Standard	0	I	II	III	IV	V
Se stock solution (μL)	0	50	125	250	375	500
Indium stock solution (μL)	500	500	500	500	500	500
Methanol (μL)	250	250	250	250	250	250

6.6.2 Optimisation of procedures for total Se analysis methods

Experimental design

A central composite Design (CCD) was developed using Design-Expert® 7.1 (Stat-Ease, Inc., Minneapolis) in order to evaluate the combined effects of nitric acid, hydrogen peroxide, sample size as well as level of methanol on total Se analysis. Based on preliminary trials, three levels of each variable (Table 6.14) were selected to cover the available process conditions.

The complete design consisted of 30 combinations i.e. sixteen cube points, four centre points in the cube, eight axial points, and two centre points in the axial. Two replications were carried out for all design points.

Table 6.14 Independent variables studied

Variable	Levels		
A. Nitric acid	1.0	1.75	2.5
B. Hydrogen peroxide	0.0	0.50	1.0
C. Sample size	0.1	0.30	0.5
D. Methanol (%)	0.0	0.50	1.0

Statistical analysis

The data were analysed statistically using One-Way ANOVA, General Linear Model ANOVA and Tukey test (MiniTab 14) and the 3D graphs for the combined effects of variables studied were constructed using Design-Expert® 7.1 1 (Stat-Ease, Inc., Minneapolis).

6.6.3 Procedures used in the validation of total Se analysis methods

In all cases, a variety of approaches were used to ensure the validity of the methods and the resulting analytical data. During the development and establishment of methods the initial approach was to measure certified standard solutions of Se. Secondly, the NIST standard reference samples were used and the results evaluated in relation to the specifications supplied with the sample (Table 6.2). The third procedure involved recovery studies in which flour samples were spiked with appropriate amounts of standard Se prior to and after extraction. Lastly, stability of extract solution was analysed by comparing 11 samples stored at 4^{o}C and room temperature. This evaluation considered storage periods of 3, 4, 5, 6, 7, 11 and 12 days.

Recoveries were calculated using the following formula:

$$\text{Recovery (percent)} = \frac{(\text{total Se in spiked sample} - \text{total Se in unspiked sample})}{\text{Se added in spiked sample}} \times 100$$

6.6.4 Calculation of total Se content to a dry weight basis

The results obtained for content of the total Se in flour, dough, noodle and breakfast cereal samples were routinely adjusted by calculation to a dry weight basis. The purpose was to facilitate the direct comparison of the results particularly for different sample types. To facilitate this, all samples analysed for total Se were also tested for moisture content. The following general equation was applied:

$$\text{Total Se content (adjusted to a constant moisture basis)} = \text{Se content (as is basis)} \times \frac{100 - \text{constant moisture figure}}{100 - \text{actual moisture of sample}}$$

In all cases the data were recalculated to a dry weight basis (where the constant moisture figure is zero) so the equation was used in the form:

$$\text{Total Se content (adjusted to a dry basis)} = \text{Se content (as is basis)} \times \frac{100}{100 - \text{actual moisture of sample}}$$

6.6.5 Duplication and presentation of analytical results for total Se contents

In the analysis of samples for total Se content, at least duplicate sub-samples of each sample were extracted. In addition, multiple analyses were performed on each extract obtained. The results of replicate analyses of each sample have been calculated and presented as mean values ± standard deviation (sd). These calculations were carried out using Microsoft® Excel 2000 software. In the evaluation of results obtained when reference materials were repeatedly analysed, the coefficient of variability of a series of values was also calculated using the following formula:

$$\text{Coefficient of variability (percent)} = \frac{\text{sd}}{\text{mean value}} \times 100$$

Chapter 7

Preliminary assessment and optimisation of procedures for extraction and quantitation of total Se from cereal based foods

The purpose of this chapter is to describe and discuss the results obtained during the evaluation and optimisation of procedures for determination of total Se in Se bio-fortified bread mix and wheat based Reference Materials.

7.1 Introduction

The two principal methods which have recently been used for the decomposition of organic materials in the analysis of Se are the oxygen flask combustion method and wet oxidation using mixed acids. It has been reported that losses due to volatilisation are negligible with the use of mixed acid at temperature of about 200°C (Zingaro & Cooper, 1974).

7.2 Evaluation of ICP-MS detection for total Se

The analysis of Se by ICP-MS is recognised to be difficult. Se has a poor signal response as a result of its relatively high first ionisation potential (9.75 eV) (Featherstone et al., 2004). Also the six available isotopes (^{74}Se (0.9%), ^{76}Se (9%), ^{77}Se (7.6%), ^{78}Se (23.6%), ^{80}Se (49.7%) and ^{82}Se (9.2%)) are subject to varying degrees of spectral interferences. The isotopes ^{74}Se, ^{76}Se, ^{78}Se and ^{80}Se all suffer from severe spectral overlap by Ar_2 (Plantz, 1996; May & Wiedmeyer, 1998; Featherstone et al., 2004). In addition, Kr, which is a potential impurity within the Ar supply overlaps with ^{80}Se and ^{82}Se. Historically, ^{77}Se and ^{82}Se have been the isotopes used for Se analysis in various food products (Featherstone et al., 2004), although interference problems are still encountered with $^{81}Br^{1}H$ for the ^{82}Se isotope while ^{77}Se can only be used for samples with relatively low chloride concentrations particularly involving low levels of $^{40}Ar^{37}Cl$, hence less interference. In the current study, both ^{77}Se and ^{82}Se isotopes were monitored and examined.

Following consideration of these issues, the ICP-MS was set up and evaluated using a series of standard Se solutions. Both isotopes chosen were analysed to see if there is any significant difference between the results obtained with these. The results are presented in Table 7.1 which shows the slope, relating to the sensitivity (Miller & Miller, 2000), the standard deviation which relates to the precision as well as the detection limit which was calculated based on the ISO regression method (ISO 11843-2:2000, ISO, Geneva). It is shown that both ^{77}Se and ^{82}Se are similarly sensitive and precise, giving detection limits of approximately about 0.414 and 0.371 µg/kg respectively.

Table 7.1 Results obtained for total selenium content using ^{77}Se and ^{82}Se
(data represent averages for five different days of experimentation)

Isotope	Slope of regression equation	Standard Deviation (SD)	Detection Limit (µg/kg)
^{77}Se	6.31E-04	8.70E-05	0.414
^{82}Se	8.08E-04	1.00E-04	0.371

In relation to the procedure for analysis a series of other variables were also considered. These included methanol addition, and concentration of nitric acid as well as the addition of hydrogen peroxide. The incorporation of methanol into the sample solution has previously been reported to enhance the sensitivity of ICP-MS (Moellmer et al, 2007). Therefore, ICP-MS was first set up and evaluated using a series of standard Se solutions, including 0, 10, 20, 30 and 40 µg/kg, with and without 1% (v/v) methanol addition.

The results obtained upon addition of 1% methanol are illustrated in Figure 7.1 and show that the slope is increased by a factor of more than two, thereby enhancing the sensitivity of the system. This slope increase is in agreement with the observations reported recently by Moellmer et al (2007). This figure also confirms the previous results (Table 7.1) that in comparing the two isotopes, Se measurement using ^{82}Se is slightly more sensitive than ^{77}Se.

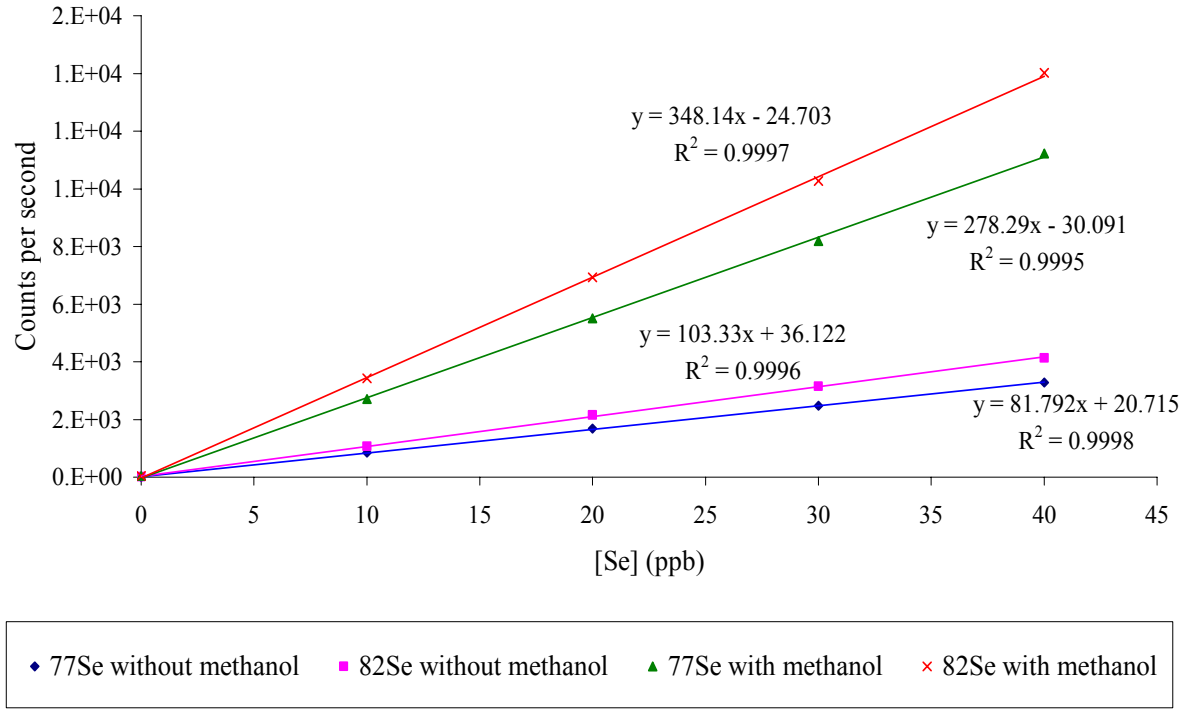


Figure 7.1 Standard curves for Se solutions analysed using ICP-MS with and without methanol addition (1% v/v)

7.3 Optimisation of microwave digestion for total selenium extraction

To facilitate the evaluation of a series of variables consideration was given to an appropriate experimental design. A Face-Centered Central Composite Design (CCF) was adopted to evaluate the combined effects of nitric acid and hydrogen peroxide as well as sample size on total Se analysis. Based on preliminary trials, three levels of each variable (Table 7.2) were selected to cover the options available for processing conditions.

The complete design consisted of 20 combinations i.e. eight cube points, six centre points in the cube, and six axial points. Two replications were carried out for all design points.

Table 7.2 Independent variables studied

Variable	Levels		
A. Nitric acid	1	1.75	2.5
B. Hydrogen peroxide	0	0.5	1
C. Sample size	0.1	0.3	0.5

The values presented in Table 7.3 illustrate the factor settings for the corresponding CCF Design. Note that this design provides three different levels for each factor.

Table 7.3 Factor Settings for CCF, designed for three variables

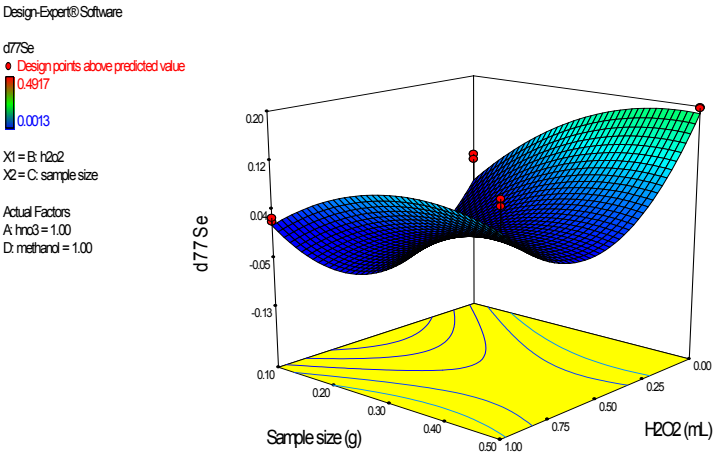
Sequence Number	Block	Nitric acid (mL)	Hydrogen peroxide (mL)	Sample size (g)
1	1	1.0	0	0.1
2	1	2.5	0	0.1
3	1	1.0	1.0	0.1
4	1	2.5	1.0	0.1
5	1	1.0	0	0.5
6	1	2.5	0	0.5
7	1	1.0	1.0	0.5
8	1	2.5	1.0	0.5
9	1	1.0	0.5	0.3
10	1	2.5	0.5	0.3
11	1	1.75	0	0.3
12	1	1.75	0.5	0.3
13	1	1.75	0.5	0.1
14	1	1.75	0.5	0.5
15	1	1.75	0.5	0.3
16	1	1.75	0.5	0.3
17	1	1.75	0.5	0.3
18	1	1.75	0.5	0.3
19	1	1.75	0.5	0.3
20	1	1.75	0.5	0.3

Using this experimental design, and the commercial bio-fortified bread mix sample, the sample digestion was studied and the results are presented in Figures 7.2 and 7.3. These display the 3D response surface graphs generated using the Design Expert® software. It is noted that in these graphs the vertical axis is the difference between the expected value and measured value. So, for example, Figure 7.2a shows the values for the differences for ^{77}Se and these values are designated as $d^{77}Se$.

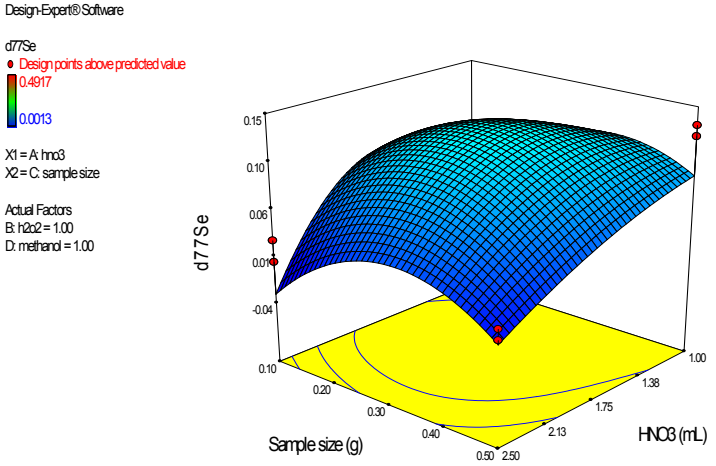
In assessing the results, the first response surface graph (Figure 7.2a) indicates that with increasing hydrogen peroxide, $d^{77}Se$ is closer to zero for each of the sample sizes evaluated. On the other hand sample size appears to have little effect (Figures 7.2a and 7.2b). Figure 7.2b indicates that there is no significant change in $d^{77}Se$ with increasing nitric acid; the combination optimum however is at the lowest end of sample size and nitric acid. Figure 7.2c demonstrates the combined effects of the levels of hydrogen peroxide and nitric acid. The optimum combination giving the lowest $d^{77}Se$ was found at the highest end for hydrogen peroxide and lowest for nitric acid respectively.

The effects of the independent variables studied (nitric acid, hydrogen peroxide and sample size) on total Se determination based on ^{82}Se isotope measurement are demonstrated in Figure 7.3. The effects of hydrogen peroxide level and sample size determined by ^{82}Se were similar to those observed for ^{77}Se. Nitric acid on the other hand resulted in little difference from the expected value (Figure 7.3c) at the lowest and highest end. Nevertheless, the optimum combination giving the lowest $d^{82}Se$ was found at the highest end for hydrogen peroxide and lowest end for nitric acid respectively.

In summary, the optimised combinations of the variables for total Se extraction in bread mix was found to be 1 mL of nitric acid, 1 mL of hydrogen peroxide, and 0.1 g sample size. Overall, these findings confirm those reported by Adams et al (2002).



(a)



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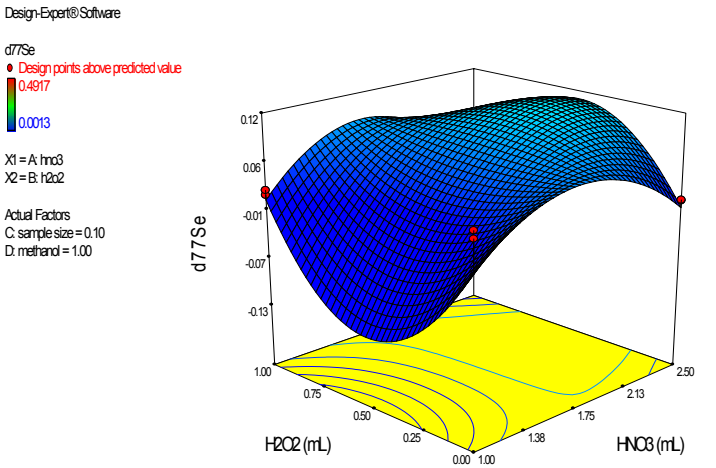
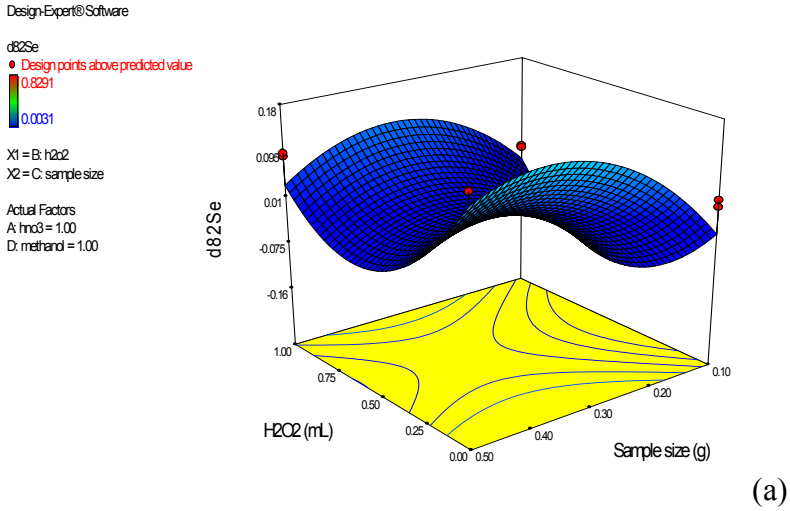
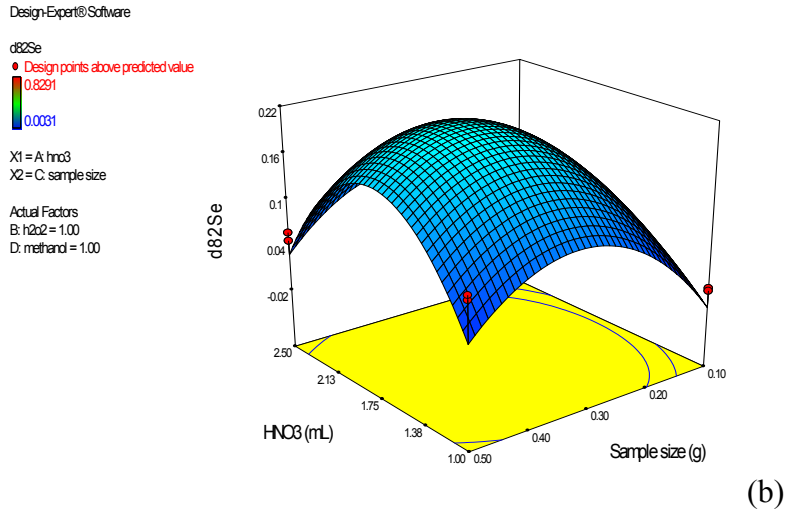


(c)

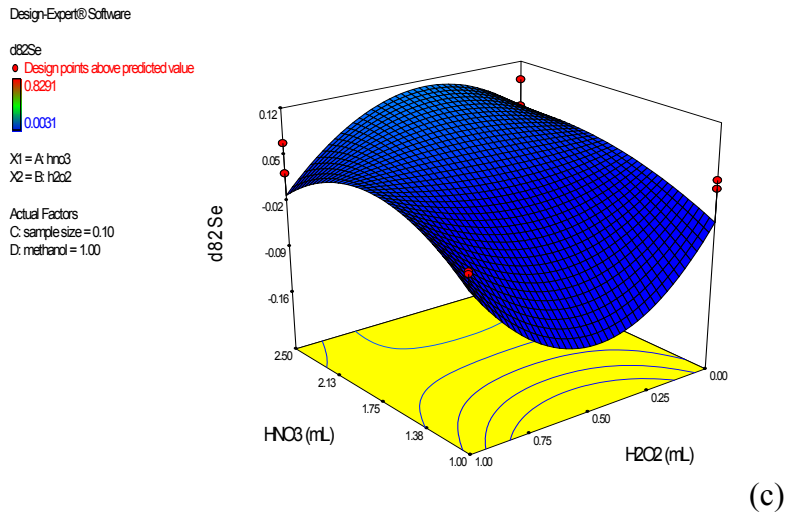
Figure 7.2 Response surface graphs of the difference between measured and expected values for ^{77}Se ($d^{77}Se$) showing the effect of: (a) sample size and hydrogen peroxide, (b) sample size and nitric acid, (c) nitric acid and hydrogen peroxide.



(a)



(b)



(c)

Figure 7.3 Response surface graphs of the difference between measured and expected values for ^{82}Se (d82Se) showing the effect of: (a) sample size and hydrogen peroxide, (b) sample size and nitric acid, (c) nitric acid and hydrogen peroxide.

7.4 Evaluation of sample extraction and validation of ICP-MS analysis of Se

The method for Se analysis of the bread-mix sample involved a series of preparation steps. Firstly the dry sample was ground using a Falling Number 3100 mill followed by careful manual homogenisation to ensure that the sub-samples weighed for digestion were representative of the original material. Subsequent microwave digestion involved a medium of 1 mL nitric acid (65% v/v) and 1 mL hydrogen peroxide (30% v/v).

Validation experiments were carried out to confirm the adequacy of the optimal settings for predicting the dependent variable values. These trials included spiking analysis in which a solution of standard Se was subjected to the same extraction conditions as samples, carried out to demonstrate if there is loss during either extraction or determination with ICP-MS. In addition, the spiking analysis is useful for the application of optimised method in foods containing a low level of Se.

The results obtained for the NIST reference samples (SRM 1567a and RM 8436) are shown in Table 7.4. Mean values obtained for both isotopes were within the certified value with good precision. The spike recoveries, for standard added prior to ICP-MS measurement range from 99 to 118% for ^{77}Se and 101-111% for ^{82}Se. These results are relatively good when considered in the context of the relatively low levels typically found in food. Nevertheless, the results for total Se analysis are highly dependent on the matrix of the sample reflected in the impact of sample weight (Figures 7.2 and 7.3). On the other hand, recovery of standard Se added prior to microwave digestion was found to be 92 to 113% and 90 to 107% for ^{77}Se and ^{82}Se respectively. This confirms that there is no significant Se loss during the microwave extraction despite the long held view that Se is relatively volatile.

Table 7.4 Validation and spiking analysis of the optimised method using NIST SRM 1567a and RM 8436

Sample	Mean (µg/g)		SD		Precision*		Recovery[#]	
	^{77}Se	^{82}Se	^{77}Se	^{82}Se	^{77}Se	^{82}Se	^{77}Se	^{82}Se
SRM (n= 6)	1.13	1.12	0.04	0.05	3.5	4.3	99 – 118[a] 92 – 113[b]	101 – 111[a] 90 – 107[b]
RM (n = 4)	1.13	1.12	0.04	0.04	3.4	3.6	ND	ND

SRM certified value = 1.1 ± 0.2 µg/g
RM certified value = 1.23 ± 0.09 µg/g
*Relative precision = SD/mean value expressed as a percentage
[#]Spike recovery = spike Se recovered / level of Se spiked as a percentage
[a] = Added prior to ICP-MS measurement
[b] = Added prior to microwave digestion
ND = Not determined

7.5 The stability of extracted samples

A further aspect of analysis that was considered was that there may be changes occurring in the chemical form of Se found in solution and that such changes might impact upon the results obtained from application of the optimized and validated procedures established in this study. To investigate this, a series of extracted samples (n=11) were stored at 4°C and room temperature and analysed at periods of 3, 4, 5, 6, 7, 11 and 12 days. The results obtained were considered in relation to those for solutions analysed immediately following digestion. In a comparison of storage conditions of 4°C and room temperature, no significant differences ($P>0.05$) were observed and there was no significant decrease in total Se in the digested samples with time for either isotope. The total Se changes with time for the two different storage conditions for the digested samples are presented in Figure 7.4. For the first seven days, there was no significant change when digested samples were stored at refrigerated condition; however there was an apparent decrease when samples were stored at room temperature (RT). After a period of seven days, there was no significant change with samples stored at RT, but a slight change with refrigerated samples.

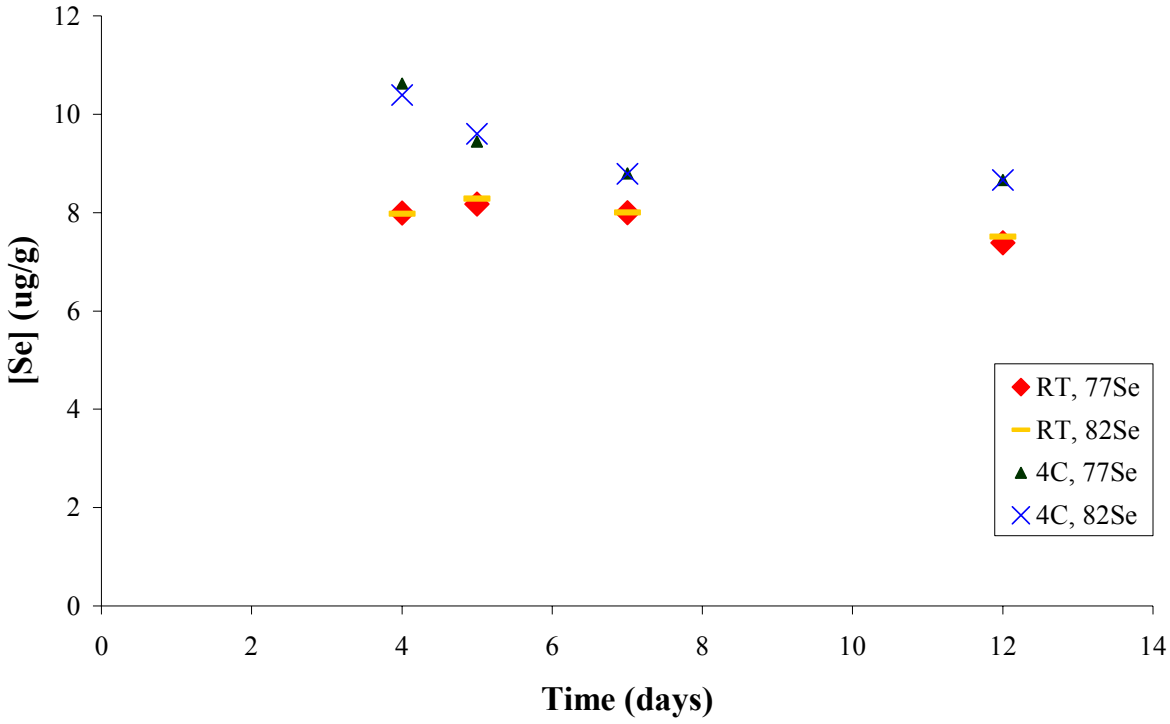


Figure 7.4 Apparent changes in Se content with time for two different two different storage conditions
(room temperature RT and refrigerated temperature 4°C)

7.6 Summary of results for establishment of analysis procedures

Following preliminary studies, approaches based upon microwave digestion for sample extraction and ICP-MS for analysis of Se content were evaluated. These procedures were optimised and then validated using wheat-based reference materials. Se was extracted using closed- vessels by microwave digestion with a mixture of nitric acid and hydrogen peroxide. The optimum conditions for Se determination in cereal based foods involved the digestion of 0.1 g samples using 1 mL of nitric acid and 1 mL of hydrogen peroxide. The addition of 1% (v/v) methanol was found to enhance the sensitivity of the ICP-MS system.

Two particular isotopes of Se (77 and 82) could be effectively employed in the analysis. In addition, there appeared to be no significant decrease in levels of total Se in the digested extracts during storage for up to twelve days under refrigeration and room temperature conditions. The results reported in this chapter have provided a procedure

considered suitable for further evaluation and application. Accordingly the next phase involved the analysis of a series of samples of the two groups of cereal grain foods selected for study.

Chapter 8

The measurement of selenium in various breakfast cereals and three different styles of Asian noodles

The purpose of this chapter is to describe and discuss the results obtained during the analysis of breakfast cereals and Asian noodles for total Se. The repeatability of the analytical procedures is also discussed.

8.1 Introduction

Cereal based foods, including breakfast cereals and Asian noodles are potentially good sources of Se. It is possible that a significant proportion of Se in the diets of many consumers may be obtained from cereal based foods especially where these are grown in areas having seleniferous soils.

Analysis of Se in biological materials has been reported to be a challenge to investigators because of its low concentration in many biological samples and losses through volatilisation during sample decomposition. Procedures for extracting Se in flour have been optimised and discussed in Chapter 7. The suitability of this optimised method for analysis of Se in various breakfast cereals and Asian noodles was further investigated and is discussed in this chapter. As there is relatively little published data for Se content, the assessment of the accuracy of the results obtained has been difficult. Nevertheless, the repeatability of results, reflected in the relative precision level, provides confidence in the experimental results.

8.2 Selenium analysis of breakfast cereals

Various breakfast cereals from major processors including Kellogg's, Uncle Tobys, Nestlé, Sanitarium and Vogel were analysed for their total Se contents. All of the samples were manufactured in Australia with the exception of Nestlé Cheerios. The results obtained for each of the samples analysed are shown in Table 8.1.

Table 8.1 Results of analysis of total selenium in breakfast cereals

Brand	Literature values (µg/g)[#]	Mean Se (µg/g)		SD		Precision*	
		^{77}Se	^{82}Se	^{77}Se	^{82}Se	^{77}Se	^{82}Se
Nestlé Cheerios	0.347	0.081	0.059	0.004	0.008	4.7	13.3
Kellogg's corn flakes	0.047 – 0.080	0.083	0.036	0.0007	0.0004	0.8	1.2
Kellogg's rice bubbles	ND	0.123	0.093	0.01	0.01	8.0	11.2
Uncle Toby's traditional oats	0.110	0.130	0.123	0.01	0.008	8.5	6.5
Sanitarium puffed wheat	ND	0.131	0.115	0.007	0.007	5.2	5.7
Home brand processed bran	ND	0.154	0.133	0.005	0.004	3.5	2.9
Kellogg's All bran	ND	0.173	0.141	0.01	0.005	5.9	3.6
Uncle Toby's Bran plus	ND	0.218	0.182	0.01	0.008	6.8	4.3
Kellogg's Nutrigrain	ND	0.244	0.183	0.008	0.007	3.5	3.9
Sanitarium Weet-Bix	0.150 – 0.260	0.245	0.218	0.01	0.01	4.7	6.3
Kellogg's Special K	ND	0.266	0.250	0.008	0.006	2.8	2.4
Vogel's Ultra bran	ND	0.373	0.378	0.007	0.011	1.7	3.0

Notes
[#] Data obtained from McNaughton & Marks (1989); in the case of Cheerios, it was obtained from USDA (2003).
* Relative precision = SD/mean value expressed as a percentage
1 Results are the mean of triplicate analyses
2 All samples were manufactured in Australia except Nestlé Cheerios, which was produced in the UK.
3 Home brand processed bran, Kellogg's Nutrigrain and Special K contain wheat flour (gluten)
4 Vogel's ultra bran contains linola (linseed) meal of 8%.

Nestlé Cheerios, which contains several cereal grains including corn (22.1%), oats (19.6%), barley (16.4%), wheat (12.1%) and rice including rice flour (7.4%), has the lowest Se content followed by Kellogg's Corn Flakes, Kellogg's Rice Bubbles, Uncle Toby's traditional rolled oats, Sanitarium Puffed Wheat, Home brand processed bran, Kellogg's All Bran, Uncle Toby's Bran plus, Kellogg's Nutrigrain, Sanitarium Weetbix, Kellogg's Special K and finally Vogel's Ultra bran. The same ranking in the results can be derived from the analyses using both the ^{77}Se and the ^{82}Se isotopes. For the majority of the samples examined, the results using ^{82}Se isotope were lower than those by ^{77}Se, however these differences were not significant for Uncle Toby's traditional oat, Kellogg's Special K and Vogel's Ultra bran. Kellogg's corn flakes could have been made from maize growing on low-Se soils. Whereas, the grain used in the Kellogg's Nutrigrain, Sanitarium Weetbix, Kellogg's Special K and Vogel's Ultra Bran would have been made from high-Se soils. The high Se content in Vogel's Ultra bran could also be due to the incorporation of linseed meal which has been claimed to be an excellent source of Se (Pfalzbot, 1996; Ingredients101, 2007).

It is noted that for those that were found to be significantly different, samples were re-analysed (Figure 8.1). The purpose of this approach was to clarify the validity of the results initially obtained as well as to investigate the reproducibility of the method employed. It was observed that analysis by either ^{77}Se or ^{82}Se isotope was not significantly different for Kellogg's Rice Bubbles and All Bran as well as Sanitarium puffed wheat. For the analysed samples, acceptable reproducibility was observed with Uncle Toby's Bran plus, Kellogg's All Bran and Rice Bubbles, as well as Sanitarium Puffed Wheat and Weetbix. Whereas, results for Home brand processed bran as well as Kellogg's Corn Flake and Nutrigrain proved more difficult to reproduce. This could be due to an uneven distribution of Se in samples in relation to the small sample size (approx. 0.1 to 0.3 g) analysed or possibly reflects some difficulty in sufficiency of extraction of Se. The later appears to be unlikely since relatively satisfactory precision values were observed except for that of Corn Flakes.

The validity of the results obtained is shown by the relative precision values which are also presented in Table 8.1. They range from as low as 0.8% to 13.3%. In the broader context of food analysis, the desirable relative precision value is usually considered to be $\leq$ 2%, nevertheless based upon the low levels of Se present, relative precision values

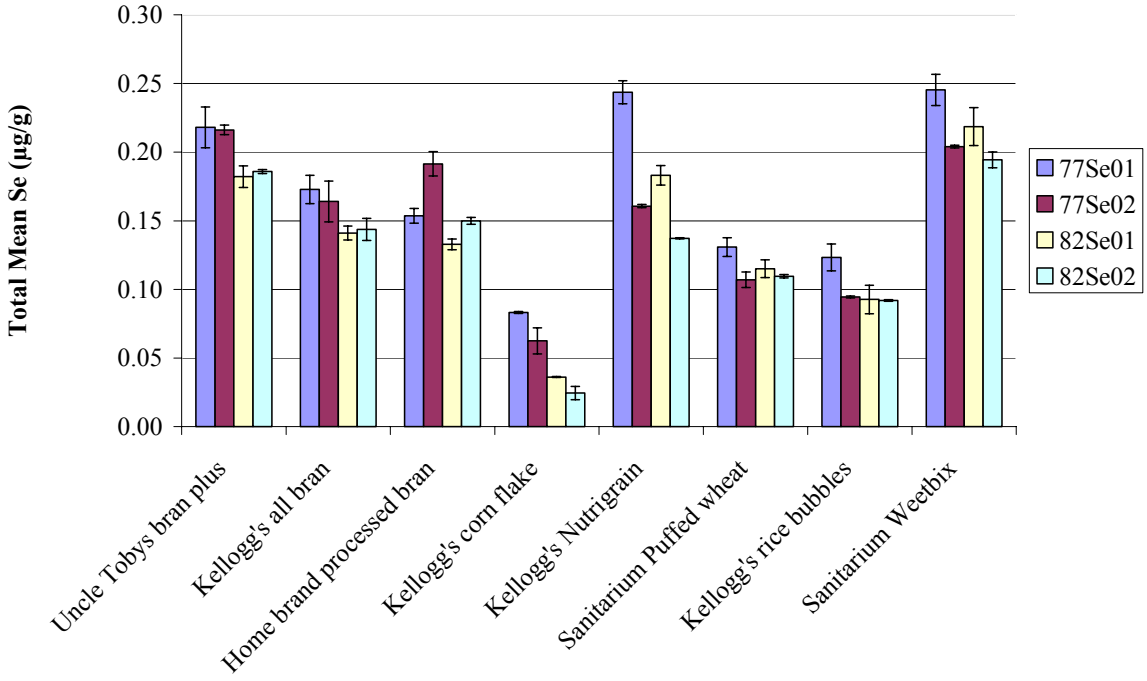
of approx. 5% are considered to be acceptable. The relative precision data observed here could be due not only to the low level of Se examined but may also reflect inadequate homogenisation of samples. In relation to the latter, the same rigorous strategies were applied for all samples; so this is not likely to be the explanation.

The results obtained for the breakfast cereals analysed here can be compared with the relatively few values available in the literature. These are included in Table 8.1 and interestingly for some products there is a wide range in the published values. The analytical results found in the current study are within the range of values reported elsewhere for each of the samples where data was found. The sample for which this was not true was Nestlé Cheerios. This exception could reflect the diverse variety of analytical procedures applied to samples even within one country. Since the value cited for Nestlé Cheerios was obtained from the USDA database (2003), large differences between the experimental value and the reported value are not surprising. This is because there is a high possibility that the sample reported in the database was manufactured in US while that analysed here was manufactured in the UK. In addition, since the soils in the US are generally known to have higher Se content compared to that in UK, therefore higher Se content would usually be expected for products from US.

Based upon the data obtained in the current study (Table 8.1), in nutritional terms, it can be estimated that consumption of one serving of breakfast cereal might provide approximately 3.0 to 18.7 percent of RDI. These figures are based upon the current Australian RDI values for male adults which is 70 µg of Se per day (NHMRC, 2005) and the serving size assumed here is equivalent to 35 g of a food. It is also noted that this discussion also assumes no loss of Se during storage and that no chemical changes are influencing the availability or chemical analysis of total Se contents.

The first conclusion from the analysis of the breakfast cereal samples is that several breakfast cereals, particularly Vogel's Ultra bran, could be considered as good sources of Se. It was also found that the levels of Se were sufficiently low in Cheerios and the Corn Flakes that reliable quantitation was challenging. The method did, however, provide relatively repeatable and reliable results over a period of some months when applied to the same samples (Figure 8.1). In addition where literature values were

available, the method used in the current study provided results comparable with the reported data (Table 8.1).



Notes For both isotopes, 01 and 02 refer to the results obtained for duplicate extracts.

Figure 8.1 Results of repeated analysis of selenium in several breakfast cereal samples using ^{77}Se and ^{82}Se isotopes

8.3 Selenium contents of Asian noodles

Samples of three different styles of Asian noodles from various countries including Australia, Hong Kong, Indonesia, Japan, Korea, Singapore, Taiwan and Thailand were analysed for their total Se contents. As background to the decision to obtain samples from processors from diverse locations, it is noted that each of the Asian countries obtain all or most of their wheat supplies through importation from US, Australia and Canada. Noodles may be made from flours milled from any of one of these sources, although the practice of blending wheats from diverse sources is common as a strategy for ensuring the desired organoleptic attributes in the noodle product. The Se content of US products is significantly higher than those from other countries due to the high available Se content in their soil. This is reflected in the literature data presented in Chapter 4, (particularly Section 4.5 and Table 4.2). In view of this fact, the Se content of noodles made from US wheat flour could be expected to be higher than those made

from other countries or a blend various countries flour. According to data reported by the Western Organisation of Resource countries (2002), one third of US exported wheat goes to Japan, one fifth to Korea, 10 to 15 percent goes to Taiwan and 7 to 10 percent goes to other countries including Hong Kong and Thailand.

The results of analysis of total Se in white salted noodles, which had been manufactured in Australia, Korea, Taiwan and Japan are presented in Table 8.2. The noodle sample from Japan showed the highest Se content, followed by products from Taiwan, Australia and Korea with no significant differences in Se content for the products from the latter two countries. These observations could be due to different sources of flour obtained for the production with the possibility that products from Australia and Korea were made using flours of similar origins.

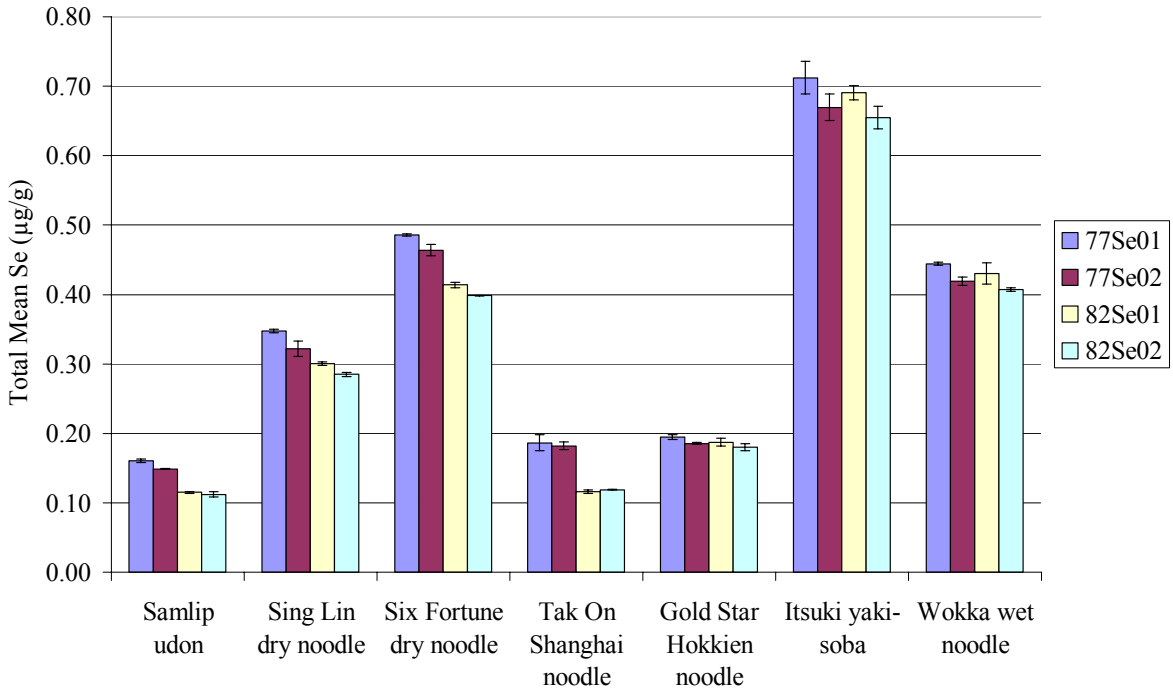
For some of the samples examined, the results using ^{82}Se isotope were significantly lower than those from ^{77}Se, excluding Couple soba (Korea), Wokka (Taiwan), Gold Star Hokkien noodle (Australia) and Itsuki yaki-soba (Japan). This may reflect some level of interference at the mass values corresponding to the Se isotopes (see Section 7.2 in Chapter 7). Subsequently, several samples including a number of those which were significantly different were re-analysed and results are presented in Figure 8.2 to verify results obtained previously as well as to investigate the repeatability of the method employed. Five out of seven samples re-examined gave satisfactory repeatability. These include Samlip udon (Korea), Tak On Shanghai noodle and Gold Star Hokkien noodle (Australia), as well as Itsuki yaki-soba (Japan) and Wokka wet noodle (Taiwan).

Table 8.2 Results of analysis of total selenium in white salted noodles

Brand	Origin	Mean (µg/g)		SD		Precision*	
		^{77}Se	^{82}Se	^{77}Se	^{82}Se	^{77}Se	^{82}Se
Hakubaku somen	Australia	0.120	0.057	0.005	0.002	4.26	3.04
Couple soba	Korea	0.136	0.113	0.004	0.001	2.72	0.87
Hakubaku soba	Australia	0.149	0.077	0.014	0.003	9.45	3.26
Samlip udon	Korea	0.160	0.115	0.003	0.001	1.65	1.14
Tak On Shanghai noodle	Australia	0.186	0.116	0.011	0.003	6.04	2.37
Gold Star Hokkien noodle	Australia	0.195	0.187	0.003	0.006	1.71	3.02
Sing Lin	Taiwan	0.347	0.301	0.003	0.003	0.74	0.85
Wokka	Taiwan	0.444	0.431	0.002	0.015	0.46	3.57
Six Fortune	Taiwan	0.485	0.414	0.002	0.004	0.34	0.95
Itsuki yaki-soba	Japan	0.712	0.691	0.023	0.010	3.28	1.46

Notes * Relative precision = SD/mean value expressed as a percentage
Number of replicates is three.
Results are expressed on a dry weight basis

In terms of reliability of the results the relative precision values range from as low as 0.3% (Six Fortune, Taiwan) to approx. 10% (Hakubaku, Australia). This is considered to be acceptable due to the low level of Se present in food products. The values are similar to those discussed earlier for the breakfast cereal products. In relation to accuracy, the analytical results might be compared to reported literature values. However, the only relevant value reported is for US wheat flour (whole grain) Se content, which is approx. 0.717 µg/g (USDA, 2003). Whilst this value appears similar to the experimental result found for the Itsuki yaki-soba sample (Japan), it also noted that wholegrain might be expected to show higher levels of Se than milled white flours as this is the typical observation for data on any vitamin or mineral component of wheat grain.



Notes For both isotopes, 01 and 02 refer to the results obtained for duplicate extracts.

Figure 8.2 Results of repeated analysis of selenium in several white salted noodle samples using ^{77}Se and ^{82}Se isotopes

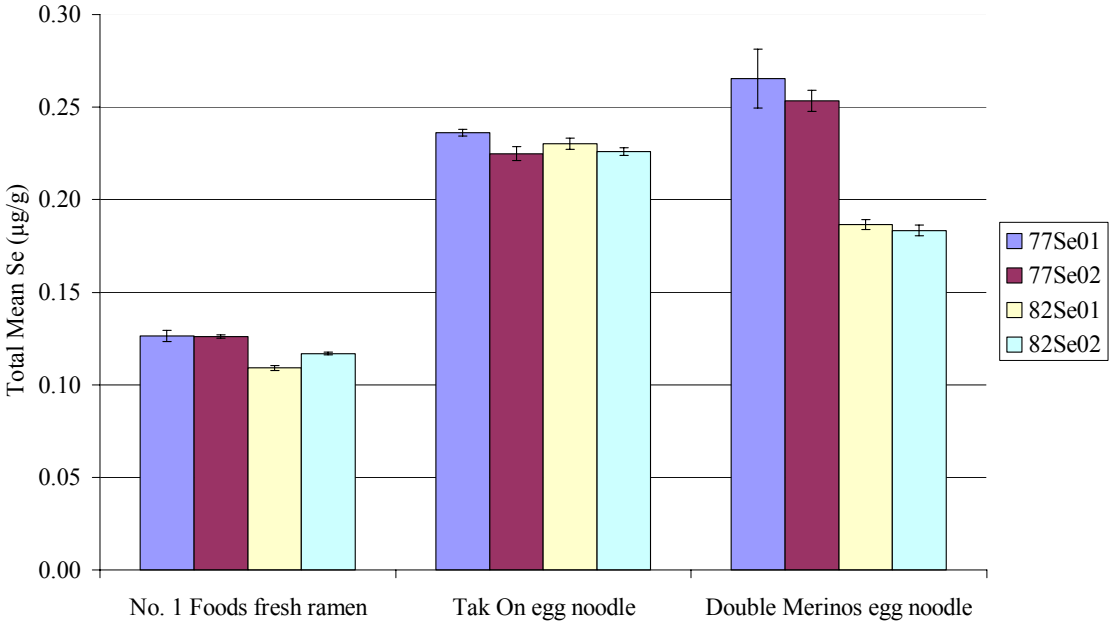
In regards to yellow alkaline noodles, each of the samples analysed was manufactured by a different Australian food manufacturer. Egg noodles produced by Tak On and Double Merinos had twice the level of Se compared to No. 1 ramen (Table 8.3). As mentioned before, Se content in wheat appears to be directly related to the Se level of the soil where the crop was grown. Therefore, substantial variation in Se contents in food products is expected even within one country.

Table 8.3 Results of analysis of total selenium in yellow alkaline noodles

Brand	Origin	Mean (µg/g)		SD		Precision*	
		^{77}Se	^{82}Se	^{77}Se	^{82}Se	^{77}Se	^{82}Se
No. 1 Foods fresh ramen	Australia	0.126	0.109	0.003	0.001	2.41	1.25
Tak On egg noodle	Australia	0.236	0.230	0.002	0.003	0.80	1.31
Double Merinos egg noodle	Australia	0.265	0.187	0.016	0.003	6.01	1.47

Notes * Relative precision = SD/mean value expressed as a percentage
Number of replicates is three.
Results were calculated on dry weight basis

A similar pattern was observed in a comparison of results based ^{82}Se isotope and ^{77}Se in which the results based on the former isotope were significantly lower than those for the latter, with the exception of the Tak On egg noodle. Consequently, all yellow alkaline noodle samples were re-investigated and results are presented in Figure 8.3 to verify data obtained previously as well as to investigate the repeatability of method employed. Satisfactory repeatability was observed with all of the samples studied. This provides further confirmation that the results achieved using the optimised method was reliable. This is reflected by the relative precision values which range from as low as 0.8% (Tak On) to approx. 6% (Double Merinos). With respect to accuracy, the only available related value reported was the Se level in bread samples from Australia. The reported data showed a range from approx. 0.09 to 0.13 µg/g (McNaughton & Marks, 2001) these being similar to the experimental result found with No. 1 Foods fresh ramen.



Notes For both isotopes, 01 and 02 refer to the results obtained for duplicate extracts.

Figure 8.3 Results of repeated analysis of selenium in several yellow alkaline noodle samples using ^{77}Se and ^{82}Se isotopes

Instant noodles from various countries including Singapore, Hong Kong, Taiwan, Australia, Korea, Thailand and Indonesia were analysed for their total Se content. The results showed that the level of Se in the samples were quite similar with the exception of instant noodles from Indonesia and Thailand which were significantly higher than for those from other countries (Table 8.4).

For the majority of samples examined, the results using ^{82}Se isotope were significantly lower than those by ^{77}Se. This might reflect specific interferences from some other component present in the food. Subsequently, a number of these samples were re-analysed and results are presented in Figure 8.4 to confirm the results obtained previously. Repeated analysis gave lower readings compared to the first batch analysis. This could be due to performance of ICP-MS or Se loss during storage of samples. Nevertheless, the differences were negligible for Maggi (Australia) and Trident (Thailand).

The relative precision values obtained vary widely from as low as approx. 0.2% (Indomie, Indonesia) to approx. 18% (Nissin, Hong Kong). Since the performance of

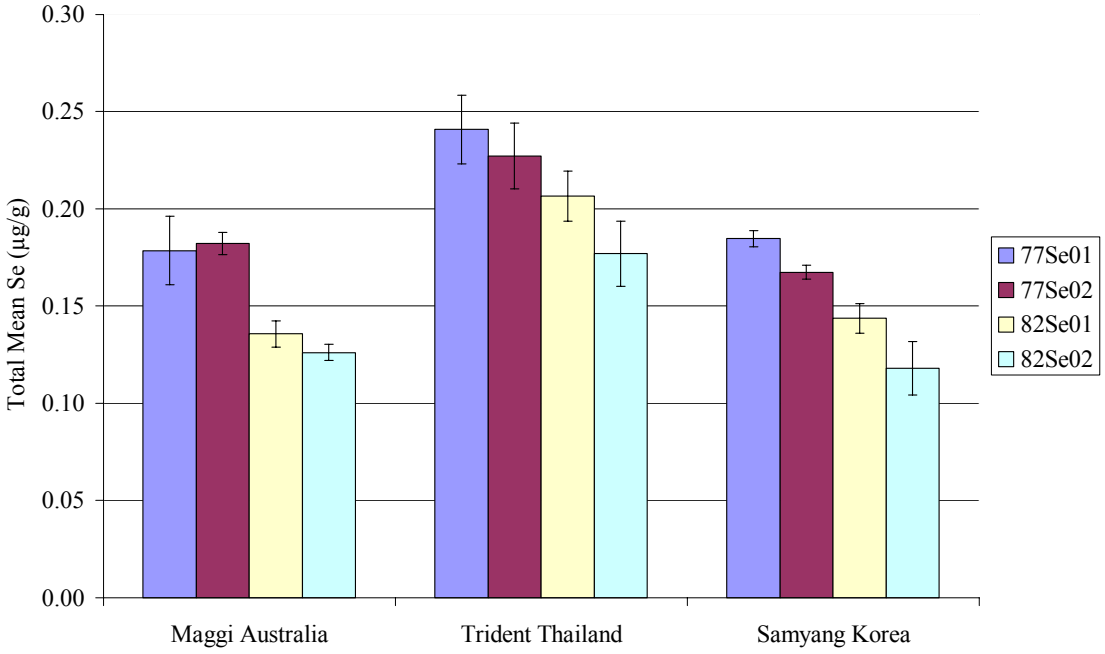
detection instrument (ICP-MS) was monitored by using Indium as internal standard, the high relative precision observed here is unlikely to be due to instrumental error.

Table 8.4 Results of analysis of total selenium in instant noodles

Brand	Origin	Mean (µg/g)		SD		Precision*	
		^{77}Se	^{82}Se	^{77}Se	^{82}Se	^{77}Se	^{82}Se
Trident	Singapore	0.146	0.077	0.011	0.005	7.74	6.86
Nissin	Hong Kong	0.155	0.104	0.014	0.019	8.82	18.33
Wei Lih	Taiwan	0.161	0.078	0.007	0.001	4.40	1.86
Maggi	Australia	0.179	0.136	0.017	0.007	9.79	4.95
Samyang	Korea	0.185	0.144	0.004	0.008	2.30	5.29
Trident	Thailand	0.241	0.207	0.018	0.013	7.34	6.23
Indomie	Indonesia	0.284	0.244	0.011	0.000	4.01	0.16

Notes * Relative precision = SD/mean value expressed as a percentage
Sample size used was approx. 0.5g for this analysis
Number of replicates is three.
Results were calculated on dry weight basis

In nutritional terms, it can be estimated that consumption of one serving of Asian noodles might provide approximately 8.1 (given by Hakubaku somen) to 101.7 (offered by Itsuki yaki-soba) percent of RDI. This figure is based upon the current Australian RDI value for male adults, which is 70 µg of Se per day (NHMRC, 2005) and the serving size assumed here is equivalent to 100 g of a food. It is noted that this discussion also assumes no loss of Se during storage and cooking prior to consumption.



Notes For both isotopes, 01 and 02 refer to the results obtained for duplicate extracts.

Figure 8.4 Results of repeated analysis of selenium in several fried instant noodle samples using ^{77}Se and ^{82}Se isotopes

8.4 General discussion and summary of results for selenium in three styles of Asian noodles

The overall conclusions from the analyses for Se in the current study are that the levels of this micronutrient in major cereal grain products analysed are relatively high in relation to the RDI values. The variability of Se content in foods could be due to the source of cereal grains or wheat flour used in production since Se content in plants depends on the Se level of soil where they were planted. Seasonal variability may also affect the Se content of foods (Reilly et al., 1993).

The results obtained by monitoring ^{82}Se were generally lower compared to those obtained with ^{77}Se. Nevertheless, the relative precision for both isotopes were typically quite good, therefore both isotopes gave reliable results. It is concluded that in most cases, the readings obtained with the two isotopes were only marginally different.

The repeatability of the method is relatively good for most food products examined. It is concluded that the optimised method employed is suitable for Se detection in cereal grain products having contents as low as 0.036 μg/g for ^{82}Se and 0.081 μg/g for ^{77}Se.

Chapter 9

The measurement and stability of selenium in fried instant noodles prepared in the laboratory

The purpose of this chapter is to describe and discuss the results obtained during the analysis of fried instant noodles for Se. Following validation, the analytical method has been applied to a study of noodles prepared under controlled conditions in the laboratory.

9.1 Introduction

Relatively little published data has been reported concerning the effect of processing steps on the apparent retention of Se in foods. In the current study two variations of fried instant noodle, specifically with and without addition of mineral salts in the formulation, were prepared in the laboratory following the method specified in Bui & Small (2007).

The specific objective of this phase of the study was to evaluate the validity of the analysis method for samples taken at various stages during processing. The approach taken was designed to establish whether there was a possibility that the ability of the method to measure all forms of Se might change as a result of the various heating and drying steps used. Some of the relevant issues are that Se is regarded as potentially volatile, that some compounds of Se may change in chemical form or react with other food components

For the purpose of this study, the flour used was biofortified bread mix. Further details on materials and preparation procedures are specified in Chapter 6 of this thesis. It is noted that although this flour is sold in Australia for bread-making, the protein content and general processing characteristics of the flour are also quite well suited to processing of instant Asian noodles.

9.2 Selenium analysis of laboratory prepared fried instant noodles

Samples of the noodles prepared in the laboratory were taken at each stage of processing. The samples were then analysed for their Se contents and the results are presented in Figure 9.1 and Table 9.1.

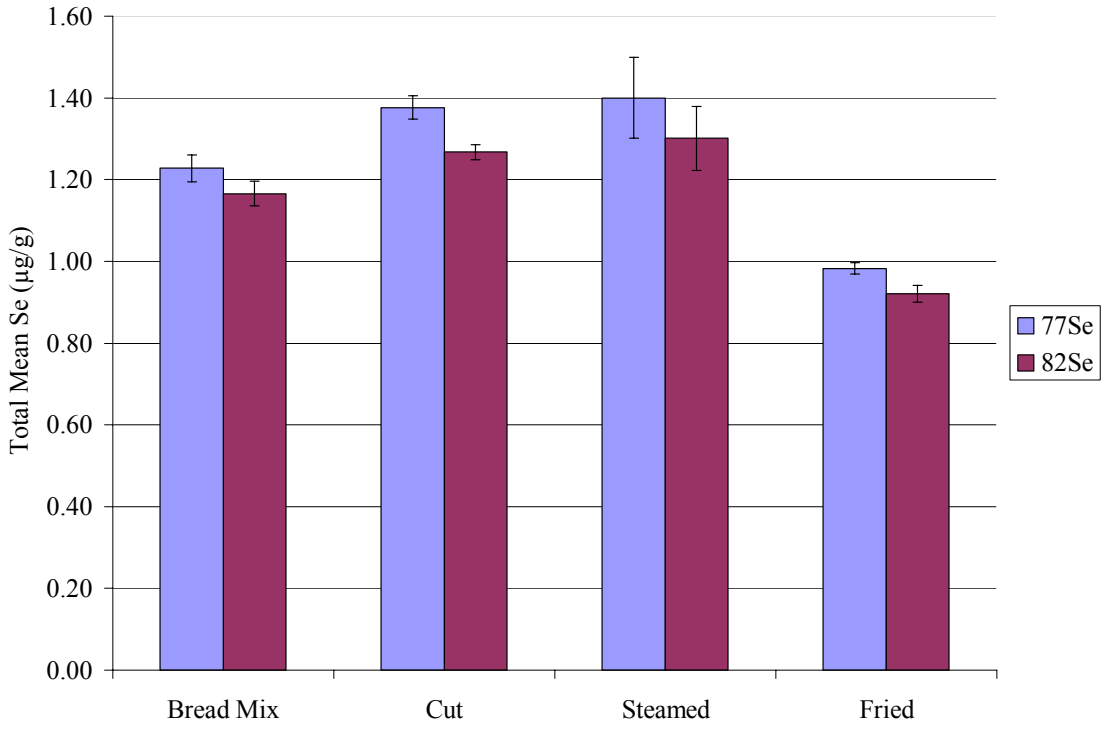


Figure 9.1 Selenium contents for noodle samples prepared in the laboratory and analysed at different stages of processing

The results demonstrate that, overall, there was no appreciable loss in Se occurring at any stage in the processing of the fried instant noodles. However, substantial losses appeared to have occurred in the final procedure of cooking the noodles, i.e frying. Unexpectedly, the Se content was marginally higher after mixing and steaming in comparison with the raw material itself. Whilst this was unexpected the observation probably reflects the low level of uncertainty associated with the ICP-MS instrumentation. The data were subjected to statistical analysis using ANOVA and the results are presented in Table 9.1.

Table 9.1 Statistical analysis of selenium contents for noodles prepared in the laboratory and sampled at different stages of processing

Processing step	Mean (μg/g)		SD		Precision*	
	^{77}Se	^{82}Se	^{77}Se	^{82}Se	^{77}Se	^{82}Se
Bread mix (raw material)	1.23[a]	1.17[a]	0.03	0.03	2.7	2.6
Dough	1.38[b]	1.27[b]	0.03	0.02	2.1	1.5
Steamed	1.40[ab]	1.30[b]	0.10	0.08	7.1	6.1
Fried noodle	0.98[c]	0.92[c]	0.01	0.02	1.4	2.2

Notes * Relative precision = SD/mean value expressed as a percentage
1 Results are the mean of triplicate analyses
2 Mean values followed by the same letter were not significantly different for each isotope ($P<0.05$)

It was noticed that the difference in terms of Se content as an effect of processing steps was significant ($P<0.05$). Se found in raw material (the bread mix) is significantly different from that of dough (after the mixing step) and steamed dough as well as fried noodle for both isotopes, with an exception that there is no significant different between bread mix and steamed dough based on ^{77}Se isotope measurement. The significantly lower Se content found in fried noodle could be due to the presence of oil in the fried noodle. This might be expected as it is known that a noodle dough will absorb significant amounts of oil during deep frying. This, in turn would result in a reduction in the amount of the raw material which is the source of the Se and thereby lowering the overall reading. This likely explanation was confirmed when the oil content in the fried noodle was analysed. Results are presented in Table 9.2.

A significant amount of fat was recovered from the noodles made in the laboratory, and the values are a little higher than those expected in commercial instant noodles. This could be due to the type of flour used and probably reflects the higher protein content of this flour. When calculations were performed to allow for the fat uptake during frying, (Table 9.3) it was observed that there is actually little Se loss. The approximate values were in the range of 3 to 4% loss during frying. This apparent loss could be due to many

reasons including suppression of Se detection with ICP-MS due to the complexity of the matrix.

Table 9.2 Fat analysis of fried instant noodle samples prepared in the laboratory

Samples	Mean (g/100g)	SD	Precision*
Fried noodle (with mineral salt in the formulation)	18.5	0.6	3
Fried noodle (without mineral salt in the formulation)	19.0	0.9	4

Notes * Relative precision = SD/mean value expressed as a percentage
1 Results are the mean of triplicate analyses

Table 9.3 Corrected selenium content of fried instant noodles after fat analysis

Fried instant noodle	^{77}Se	^{82}Se
Mean value (μg/g)	0.98	0.92
Corrected mean value (μg/g)	1.20	1.13
Se loss	3%	4%

Notes 1 Corrected mean value = mean value/ (1 - fat content per gram)
2 Se loss = (corrected mean value – bread mix Se content) * 100%

In summary it can be concluded that Se is well retained in instant noodles following its processing steps which involved steaming and frying. This is consistent with the various observations showing that Se is relatively stable. ICP-MS is effectively able to measure the total Se contents and the traditional view that Se is volatile is not applicable to the preparation of instant noodles.

9.3 Evaluation of potential interferences of salt level on Selenium analysis of fried instant noodles prepared in the laboratory

One further issue in Se analysis was investigated using samples of noodles made under controlled conditions in the laboratory. In the manufacture of most brands of instant noodles, alkaline salts are added and as part of a recent investigation of nine commercial instant products the pH was reported to have varied from 6.4 to 8.2 (Bui & Small, 2006). This indicates that varying levels of the alkaline ingredients were probably incorporated into the formulations. In relation to the current study, there is then the possibility of interference in the ICP-MS analysis due to varying levels of mineral salts in the food matrix. Accordingly experiments were designed to clarify the issue of the effect of mineral salts on the Se analysis procedure.

For this purpose, initially a solution of mineral salts with 100 μg/kg concentration was prepared by using six parts (by weight) of potassium carbonate (K_2CO_3) and four parts of sodium carbonate (Na_2CO_3). These mineral salts, in this ratio, are commonly applied in the making of instant noodles. The freshly prepared solutions with and without the presence of mineral salts were then spiked with 10 μg/kg Se (IV) and analysed using ICP-MS and the results are presented in Table 9.4.

Table 9.4 Effect of mineral salts addition on ICP-MS detection

Samples	Without mineral salts		With mineral salts	
	^{77}Se	^{82}Se	^{77}Se	^{82}Se
1	9.55	9.63	9.15	9.28
2	9.54	9.67	9.16	9.30
Mean	9.55	9.65	9.15	9.29
SD	0.005	0.03	0.004	0.01
Precision*	0.05	0.3	0.05	0.2

Notes * Relative precision = SD/mean value expressed as a percentage
Results in units of μg per g

It is noticeable that addition of mineral salts appeared to have some effect in suppressing Se detection. Readings obtained with mineral salts were generally lower by approx. 4% either and similar observations were made using both of the isotopes, ^{77}Se and ^{82}Se. This also showed that complexity of matrix will affect Se detection in organic materials. This was also reflected earlier during the optimisation of method where effects of sample size, nitric acid and hydrogen peroxide were studied (Chapter 7). Despite the minor concerns raised in relation to the impact of the alkaline salts, it is noted that the results have low values for relative precision.

To further clarify the influence of the salts, instant noodles were prepared with and without the addition of the salts. Samples of the product were collected at two stages: after cutting and also following frying and then analysed using the optimized microwave digestion-ICP-MS approach. It was consistently observed that the Se readings were lower for samples into which no mineral salts had been incorporated (Table 9.5). This may appear contradictory to the initial findings that showed higher recovery in solutions without the present of mineral salts. In this case however, the differences between the results either with or without addition of mineral salts were not significant ($P<0.05$) except for cut noodle based on ^{77}Se isotope analysis.

Table 9.5 Effect of mineral salt addition to instant noodle on ICP-MS detection

Samples	^{77}Se		^{82}Se	
	With	Without	With	Without
Dough (cut)	1.38 ± 0.03	1.29 ± 0.04	1.27 ± 0.02	1.24 ± 0.04
P-value	0.038		0.374	
Fried	0.98 ± 0.01	1.02 ± 0.02	0.92 ± 0.02	0.96 ± 0.02
P-value	0.077		0.102	

Note Results are the mean of triplicate analyses and are expressed as mean ± sd in units of μg per g

9.4 Summary of results for selenium analyses in instant noodles

In the context of the consistent results having good precision?, obtained for the Se contents of a series of commercial instant noodle products, the current phase of this study has applied the extraction and analysis procedures to a study of products made in the laboratory. These trials have shown that there is an apparent loss of Se during the deep frying of instant noodles. However when the substantial uptake of fat during deep frying is taken into account, there is no significant change in the Se content from original ingredients to the final product.

A further series of experiments also showed that there is not a strong impact of the food matrix in the analysis of these products?. The presence of varying levels of addition of mineral salts does not affect Se measurement significantly. In addition, potential concerns due to the possible changes which might occur in the chemical form of Se in either the food during processing as well as in extracts prepared for analysis do not have a strong impact on the results obtained. Overall the usefulness of the procedures for total Se has been demonstrated and confirmed.

Chapter 10

General discussion and conclusions

The purpose of this chapter is to summarise the results obtained during the current study, draw final conclusions and make recommendations for further research into Se in cereal grain based foods.

10.1 Introduction

In developing this project a survey of the literature indicated that Se levels in food can vary widely, not only between countries but also between regions within a country. Se levels in cereal grain products are considered to be relatively low. Nevertheless, considering the quantities in which cereal grain products are consumed, these are therefore a significant source of Se in a diet of many people. Further issues are that there is a lack of published data in term of Se content in samples of interest. This becomes a challenge for those seeking to compare data and considering the accuracy of results obtained. Nevertheless, the repeatability of results which is reflected by the relative precision level would provide confidence to experimental results. In addition, it is also important to know if Se is stable upon processing.

Among the available methods for Se analysis in cereal grain foods, it has been reported that the most recent analyses have been performed by adopting microwave technology and heating block using concentrated acids, either as a single type of acid or a mixture of various acids, followed by detection using ICP-MS.

The results described in this thesis fall into four broad areas. These are:

1. Optimisation and validation of Se analysis procedures
2. Analysis of breakfast cereals and Asian noodles samples
3. Studies of the stability of Se in fried instant noodles prepared in the laboratory under controlled conditions

The results for each of these are now reviewed as a basis for presenting the primary conclusions of this project as well as a discussion of areas recommended for further research.

10.2 Optimisation and validation of selenium analysis procedures

Whilst method development was not the primary emphasis of this project, considerable effort was made to optimise and validate the suitable method for total Se analysis. Firstly, the preferred detector, ICP-MS was set up and evaluated using a series of certified standard Se solutions. Both ^{77}Se and ^{82}Se were analysed to see if there was any significant difference between the results obtained with the two isotopes. It was found that both ^{77}Se and ^{82}Se are similarly sensitive and precise giving about 0.414 and 0.371 µg/kg for detection limit respectively. Other parameters for the analysis were also considered. These include methanol addition and concentration of nitric acid as well as hydrogen peroxide. Of particular note was the finding that with the addition of 1% methanol, the slope of the calibration curve is increased by a factor of more than two, thereby substantially enhancing the sensitivity of the system.

Microwave digestion for total Se extraction was also optimised by adopting a Central Composite Design to evaluate the combined effects of nitric acid, hydrogen peroxide as well as sample size on total Se analysis. The optimum combinations of the variables for total Se extraction in bread mix were achieved by digesting 0.1 g of sample using combination of 1 mL of nitric acid and 1 mL of hydrogen peroxide.

This optimised method was then validated using Reference Materials including SRM 1567a and RM 8436 along with spiking analysis performed to establish whether any significant loss was occurring during microwave extraction or discrepancies with ICP-MS detection; this reflected the matrix effects on Se analysis. The mean values obtained for both isotopes measured were within the certified range and demonstrated good precision. The spike recovery ranged from 99 to 118% when spiking was performed prior to ICP-MS detection, while the recovery rate was 90 to 113% when spike was added before microwave digestion. This shows that Se analysis may be partly dependent on the matrix but there is no significant loss with microwave digestion.

Extracted samples were also evaluated in terms of their stability. For this, samples were stored for periods of 3, 4, 5, 6, 7, 11 and 12 days at 4°C and also at room temperature. There was no significant decrease in total Se in the digested samples with time for either of the two storage conditions. However, for the first seven days, an apparent decrease was observed with samples stored at room temperature. After the period of seven days, no significant change was noticed with samples stored at room temperature but a slight change was observed with refrigerated samples.

In can be concluded that both isotopes measured, namely ^{77}Se and ^{82}Se are suitable for use in Se analysis in cereal grain foods. The addition of 1% methanol improves the sensitivity of total Se detection by ICP-MS. Digested samples are relatively stable over the period of time studied (12 days) either stored in RT or under refrigerated conditions. The optimised method has been validated and gave relatively reliable results.

10.3 Analysis of breakfast cereals and Asian noodles samples

The suitability of this optimised method for the analysis of Se in various breakfast cereals and Asian noodles was further investigated and discussed. A series of breakfast cereals from major manufacturers including Kellogg's, Uncle Tobys, Nestlé, Sanitarium and Vogel were analysed for their total Se contents. All of the samples were manufactured in Australia with the exception of Nestlé Cheerios. This latter product was found to have the least Se content of all the products analysed. This was followed by Kellogg's Corn Flakes, Kellogg's Rice Bubbles, Uncle Toby's traditional oats, Sanitarium Puffed Wheat, Home brand processed bran, Kellogg's All Bran, Uncle Toby's Bran plus, Kellogg's Nutrigrain, Sanitarium Weetbix, Kellogg's Special K and finally Vogel's Ultra bran derived from the analysis using both ^{77}Se and ^{82}Se isotopes.

In regards to Asian noodles, three different styles of Asian noodles from various countries including Australia, Hong Kong, Indonesia, Japan, Korea, Singapore, Taiwan and Thailand were analysed for their total Se contents. For white salted noodles, products of Japan had the highest Se content, followed by noodles from Taiwan, Australia and Korea with insignificant difference in Se content of the products from the latter two countries. Whereas, for yellow alkaline noodles (Australia made), egg noodles produced by Tak On and Double Merinos had twice the amount of Se compared to No.

1 ramen. Lastly, instant noodles from various countries including Singapore, Hong Kong, Taiwan, Australia, Korea, Thailand and Indonesia were analysed and results showed that the level of Se in the samples analysed were relatively similar with the exception of the samples of instant noodles from Indonesia and Thailand which were significantly higher than samples studied from the other countries.

The difference in Se content observed here most likely related to the Se content of soil where the raw materials were cultivated, in addition to the various abilities of different cereal species to take up from the soil and accumulate Se in the grain. It is noted that the in the current study, a limited number of samples were analysed and that in each case a single batch run was sampled for analysis. It might be expected that some variation would be observed if a single product were sampled and analysed over time. Further studies might usefully investigate these issues. Nevertheless, the results obtained in this study demonstrate that different products do vary in their total Se content.

The reliability of results is revealed by the relative precision values. For instance, for breakfast cereals, the relative precision of results obtained range from as low as 0.8% to 13.3%. Whereas, for white salted noodles, they ranged from as low as 0.3% (Six Fortune, Taiwan) to approx. 10% (Hakubaku, Australia). For yellow alkaline noodles, they ranged from as low as 0.8% (Tak On) to approx. 6% (Double Merinos). Finally, they vary widely from as low as approx. 0.2% (Indomie, Indonesia) to approx. 18% (Nissin, Hong Kong) for fried instant noodles. The desirable relative precision value is usually $\leq$ 2%, nevertheless considering the low level of Se present, these relative precision values are considered to be acceptable.

In terms of repeatability of method, acceptable results were observed with Uncle Toby's Bran plus, Kellogg's All Bran and Rice Bubbles, as well as Sanitarium Puffed Wheat and Weetbix. Whereas, results for Home brand processed bran as well as Kellogg's Corn Flakes and Nutrigrain showed poorer reproducibility. Similarly the results for noodle samples were quite repeatable. It may be that of the limitations of the microwave digestion system is that the maximum size of sample which can conveniently be analysed is relatively small. As a result there could be an uneven distribution of Se in samples in relation to the small sample size (approx. 0.1 to 0.3 g).

In nutritional terms, it has been estimated that consumption of one serving of breakfast cereal might provide approximately 1.8 to 18.9 percent of RDI. This figure is based upon the current Australian RDI values for male adults which are up to 70 µg of Se per day (NHMRC, 2005) and the serving size assumed here is equivalent to 35 g of a food. Whereas, Asian noodles for instance might provide approximately 8.1 (given by Hakubaku somen) to 101.7 (offered by Itsuki yaki-soba) percent of RDI per serving size. The serving size assumed here is equivalent to 100 g of a food.

10.4 Studies of the stability of selenium in fried instant noodles prepared in the laboratory under controlled conditions

Samples of fried instant noodles prepared in the laboratory were taken at each stage of processing. The samples were then analysed for their Se contents. The results demonstrate that, overall, there was no considerable loss of Se during the processing of fried instant noodles. The significantly lower Se content in fried noodles was found to reflect the oil uptake of noodles during deep frying. The corrected values obtained showed that approximately 3 to 4% was lost during this frying step. This agrees with the studies that found no effect of most thermal processes tested on Se content of wheat or white flour (Hakansson et al., 1987; Lyons et al., 2003; Lyons et al., 2005a)

In addition, effect of mineral salts on Se analysis was also assessed. A solution of mineral salts with 100 µg/kg concentration was prepared by using six parts of potassium carbonate (K_2CO_3) and four parts of sodium carbonate (Na_2CO_3). These mineral salts and their ratios are commonly applied in the making of instant noodles. The freshly prepared solutions were then spiked with 10 µg/kg Se (IV) and analysed by using ICP-MS. It is noticeable that addition of mineral salts suppressed Se detection. Readings obtained with mineral salts were generally lower by approx. 4% either by using ^{77}Se or ^{82}Se isotope. This also showed that complexity of matrix will affect Se detection in organic materials. This was also reflected earlier during the optimisation of method where effects of sample size, nitric acid and hydrogen peroxide were studied. These results were reliable as reflected by the low relative precision (0.05 to 0.2%).

Furthermore, instant noodles were prepared with and without salts. Samples after cutting and frying were collected and analysed. It was observed that Se reading was

lower without the addition of mineral salts. The differences between the results either with or without addition of mineral salts were not significant ($P<0.05$) except for cut noodle based on ^{77}Se isotope analysis.

10.5 Possible areas for future research

This study has concentrated on total Se in breakfast cereals and three different styles of Asian noodles and upon the effect of fried instant noodle processing steps. It would be of value to extend this work to other flour and cereal grain foods. One specific example is of steamed breads which represent a staple food in parts of Asia including northern China. Typical formulations contain ingredients which would be expected to result in alkaline conditions in the product. Accordingly studies of these products may also provide further insights on the nutritional value of flour based foods. Such work may also lead to a reassessment of our understanding of the adequacy of nutrient intakes and the impact of processing.

In addition, based upon the potential of enhanced intakes and the possible fortification of foods influencing the health and longevity of consumers, it is important to have reliable speciation methods which discriminate between the various forms of Se present in the diet. It is also crucial to know if different chemical form can be used as fortifying agents along with the stabilities of the added compounds during processing and storage of food ingredients and products.

In the context of nutritional value, milling of the wheat grain is known to be important. Although this issue was outside the scope of the current study, further research may now be warranted. It is well established that milling extraction rates directly affect the content of vitamins and other nutrients in white flours (Ranhotra, 1994; Gregory, 1996; Fujino et al., 1996; Lyons et al., 2005a). There is a lack of recent published data on vitamin contents of milled flours of varying extraction rates. This may be a significant issue for further research because there have been many technological developments in grain milling since the early data was published. Recent advancements in milling technology and practice may have allowed greater nutrients retention in flours (Stenvert, 1995; Forder, 1997).

There are further reasons for reinvestigation of the impact of milling on Se contents in flour. One of these is the increasing evidence that Se is important nutritionally and the ongoing reassessment of the dietary sources of this nutrient and the desirability of fortification for the population as a whole especially for the areas where Se levels are low.

In conclusion, there have been rapid developments in our knowledge over recent years in the areas of the importance of Se nutritionally as well as its analysis in various foods. However, there are few studies on Se in cereal based foods and the effect of their processing steps and storage. The research reported here is the first investigation of total Se in Asian noodles and selected processing steps of these products. It is hoped that this work might form the basis of further studies of Asian noodles and other food products, ultimately leading to the enhancement of their nutritional value. It appears that much remains to be done to ensure adequate nutrition for our expanding world population.